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(21) International Application Number: PCT/US93/08808 (22) International Filing Date: 16 September 1993 (16.09.93) (30) Priority data: <table border="0"> <tr> <td>946,238</td> <td>16 September 1992 (16.09.92)</td> <td>US</td> </tr> <tr> <td>029,335</td> <td>4 March 1993 (04.03.93)</td> <td>US</td> </tr> <tr> <td>040,510</td> <td>31 March 1993 (31.03.93)</td> <td>US</td> </tr> </table> (71) Applicant: CREATIVE BIOMOLECULES, INC. [US/US]; 45 South Street, Hopkinton, MA 01748 (US). (72) Inventors: KUBERASAMPATH, Thangavel ; Six Spring Street, Medway, MA 02053 (US). RUEGER, David, C. ; 19 Downey Street, Hopkinton, MA 01748 (US). OPPER-MANN, Hermann ; 25 Summer Hill Road, Medway, MA 02053 (US). PANG, Roy, H., L. ; 15 Partridge Road, Etna, NH 03750 (US). COHEN, Charles, M. ; 98 Winthrop Street, Medway, MA 02053 (US). OZKAYNAK, Engin ; 44 Purdue Drive, Milford, MA 01757 (US). SMART, John, E. ; 50 Meadow Brook Road, Weston, MA 02193 (US).		946,238	16 September 1992 (16.09.92)	US	029,335	4 March 1993 (04.03.93)	US	040,510	31 March 1993 (31.03.93)	US	(74) Agent: KELLEY, Robin, D.; Testa, Hurwitz & Thibault, Exchange Place, 53 State Street, Boston, MA 02109 (US). (81) Designated States: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
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(54) Title: MORPHOGEN-INDUCED LIVER REGENERATION**(57) Abstract**

Disclosed are therapeutic treatment methods, compositions and devices for maintaining liver function in a mammal, including means for regenerating lost or damaged hepatic tissue, means for enhancing viability and integration of hepatic tissue and organ transplants, and means for correcting liver function deficiencies, including means for enhancing diminished liver function due to tissue injury or disease. The methods, compositions and devices on this invention all provide a therapeutically effective morphogen concentration to the hepatic cells to be treated. Also disclosed are methods and compositions useful in a gene therapy or drug delivery protocol for correcting a protein deficiency in a mammal.

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Morphogen-Induced Liver Regeneration

FIELD OF THE INVENTION

The present invention relates generally to liver treatment methods.

5

BACKGROUND OF THE INVENTION

The present invention relates to methods and compositions for regenerating lost or damaged liver tissue in vivo and to methods and compositions for maintaining normal liver function which may be reduced or lost as a result of such tissue damage. The invention further relates to methods and compositions for correcting one or more liver function deficiencies in a mammal, particularly a human.

15

The liver is the largest visceral organ in the body and consists of two main lobes, a larger right lobe and a smaller left lobe. The right lobe also contains two smaller segments referred to as the caudate and quadrata lobes. The liver has a dual blood supply, consisting of the hepatic artery and the portal vein. The hepatic lymphatics drain principally into lymph nodes of the porta hepatis and celiac axis.

20

The liver is responsible for a wide variety of functions, broadly characterized as metabolic, storage, synthetic, catabolic and excretory. Specifically, the liver is the central organ of glucose homeostasis, responsible for both storing excess blood glucose as glycogen and restoring blood glucose by glycogenolysis

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and gluconeogenesis and by converting free fatty acids to triglycerides and lipoproteins. The liver also stores triglycerides, iron, copper and lipid-soluble vitamins and synthesizes many of the binding proteins for iron, copper and Vitamin A.

In addition, most serum proteins, with the exception of immunoglobulins, are synthesized in the liver, including albumin, the principal source of plasma oncotic pressure, blood clotting factors such as prothrombin, fibrinogen and Factor VIII, as well as complement and other acute phase reactants involved in an immune response. The liver also functions as a catabolic site for hormones, serum proteins, and other endogenous proteins, as well as acting as the detoxification site for foreign compounds, including drugs (pharmaceuticals), industrial chemicals, environmental contaminants, and various bacterial metabolism byproducts. Finally, the liver excretes bile, which provides a repository for the products of heme catabolism and also is vital for fat absorption in the small intestine.

Not surprisingly, liver function disorders, whether resulting from a particular protein deficiency or from hepatic tissue damage and/or loss, has serious and far-reaching consequences. For example, reduced albumin levels in chronic liver disease contribute to the development of edema and ascites; liver failure also is characterized by severe and often life-threatening bleeding, due to the reduced production of essential blood clotting factors. Hepatic failure also can induce neurological dysfunction, characterized broadly as hepatic encephalopathy, as well as associated renal failure, jaundice, pulmonary complications, and a host of disorders associated with hormonal imbalances.

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Unlike most other organs in the body the liver has a defined regenerative capacity following hepatic tissue damage or cell death. Specifically, while

5 hepatocytes do not proliferate actively following fetal and post natal liver growth, normally quiescent hepatocytes do divide in response to cell death or loss of liver tissue. However, where tissue damage is extensive and/or chronic, permanent tissue damage can

10 result, reducing the organ's viability and functional capacity. Permanent hepatic tissue damage typically is characterized by extensive necrosis and/or fibrogenesis or scarring (cirrhosis). Another source of nonreparative damage results from hepatic neoplasms and

15 metastatic carcinomas.

Where either the mass of liver cells is sufficiently diminished or their function sufficiently impaired, hepatic failure ensues. The etiology of

20 hepatic failure may be metabolic (e.g., altered bilirubin metabolism or fatty acid storage), infectious (e.g., induced by viral hepatitis, hepatic schistomiasis, syphilis, or ascariasis), toxic (e.g., induced by ethanol, ammonia, phenol, and other

25 environmental toxins, fatty acids, drugs and/or their metabolites), autoimmune, ischemic or nutritional (e.g., alcoholic liver disease).

Another source of hepatic failure results from

30 malignant tumors. The tumor cells may be derived from hepatic tissue cells (as in hepatocellular carcinoma, bileduct carcinomas, hepatoblastomas or hemangiosarcoma) or may be derived from distant tissue as part of a metastatic cancer. In fact, metastatic

35 cancers are by far the most common malignant neoplasms of the liver, most notably derived from cancers of the gastrointestinal tract, breast and lung.

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Another source of diminished liver function arises from hepatic protein deficiencies, which may result from a genetic defect (so called "inborn errors of metabolism") or may be induced by, for example, a pharmaceutical, infectious agent byproduct, or the like. For example, hemophilia is believed to be associated with diminished Factor VIII production, Wilson's disease, a copper metabolism disorder, is associated with deficient ceruloplasmin production by the liver, altered albumin production affects bilirubin metabolism, and α_1 -antitrypsin deficiency, normally produced in the liver, can result in fatal neonatal hepatitis.

15

To date, the only viable treatment for hepatic failure or for patients at risk for hepatic failure due to, for example, chronic acute hepatitis, biliary atresia, idiopathic cirrhosis, primary biliary cirrhosis, sclerosing cholangitis, inborn errors of metabolism or malignancy, is liver transplantation. To date, liver transplantation also is the only viable alternative for correcting significant liver function deficiencies that result from inborn errors of metabolism. Liver transplantation as a treatment method suffers from donor scarcity, particularly of pediatric livers, technical surgical complexity, postoperative complications including organ rejection, and continuing difficulties in maintaining organ viability throughout the transplant process.

30

Selective cell transplantation of only those parenchymal elements necessary to replace lost function has been proposed as an alternative to whole or partial organ transplantation that avoid major surgery with its attendant blood loss, anesthetic difficulties, and complications (P.S.Russell, Ann. Surg. 201(3), 255-262

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(1985). Replacing only those cells which supply the needed function reduces problems with passenger leukocytes, antigen presenting cells, and other cell types which may promote the rejection process. The ability to expand cell numbers with proliferation of cells in culture, in theory, allows autotransplantation of one's own tissue. In addition, transplantable cells may be used as part of a gene therapy to correct a liver protein deficiency, and/or as in vivo drug delivery vehicles. WO88/03785 published June 2, 1988, and WO90/12640 published November 1, 1990, both describe methods for attaching hepatocytes to matrices and implanting the matrices at sites in vivo that are capable of providing the cells with adequate nutrition or gas exchange, such as within mesentery folds or the omentum. To date, the existing protocols suffer from a variety of limitations. Typically, partial hepatectomy is required to stimulate cell proliferation of the synthetic tissue in vivo. In addition, cell implantation typically is accompanied by significant cell loss, requiring a substantial seed cell population for implantation, which may further require lengthy in vitro incubation periods. The delay in in vivo integration of the implanted cell-matrix structure also places significant restrictions on the matrix scaffold composition. Finally, the implanted cell-matrix structures also are at risk for destruction by the implant host's immune response mechanisms.

It is an object of this invention to provide methods and compositions for regenerating lost or damaged hepatic tissue in vivo in an existing liver without requiring organ or tissue transplant. Another object is to provide means for maintaining normal liver function following hepatic tissue injury or in anticipation of such injury. Another object is to

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provide means for enhancing or increasing a depressed liver function level which may result from a tissue injury or disease. Still another object is to provide methods and compositions for correcting a liver
5 function deficiency in a mammal. Yet another object is to provide gene therapy protocols and compositions useful for correcting a protein deficiency in a mammal. Yet another object is to enhance integration of a liver tissue implant. These and other objects and features
10 of the invention will be apparent from the description, drawings and claims which follow.

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Summary of the Invention

The present invention provides methods and compositions for maintaining liver function in a mammal. The invention provides means for correcting one or more liver function deficiencies in a mammal that may arise, for example, from an inborn metabolism defect, and means for regenerating lost or damaged hepatic tissue in a mammal, including means for protecting the tissue from damage thereto. The invention also provides means for enhancing the viability of a hepatic tissue or organ to be transplanted and means for enhancing the integration of the transplanted tissue. The methods and compositions of this invention include providing to hepatic cells a therapeutically effective concentration of a morphogenic protein ("morphogen", as defined herein) upon hepatocellular injury, or in anticipation of such injury, or following diagnosis of a liver function defect in a mammal, for a time and at a concentration sufficient to maintain or regain liver function in vivo.

In one aspect, the invention features compositions and therapeutic treatment methods that include administering to a mammal a therapeutically effective amount of a morphogenic protein ("morphogen"), as defined herein, upon hepatocellular injury, or in anticipation of such injury, or following diagnosis of a liver function deficiency, for a time and at a concentration sufficient to maintain normal and/or to regain lost liver function in vivo, including regenerating lost or damaged hepatic tissue, and/or inhibiting additional damage thereto. The morphogens described herein also are capable of enhancing the level of a liver function which may be depressed as a result of a tissue injury or disease.

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- In another aspect, the invention features compositions and therapeutic treatment methods for maintaining liver function in a mammal in vivo which include administering to the mammal, upon
- 5 hepatocellular injury or in anticipation of such injury, or following diagnosis of a liver function deficiency, a compound that stimulates in vivo a therapeutically effective concentration of an endogenous morphogen within the body of the mammal
- 10 sufficient to increase or enhance the level of a depressed liver function, and/or to maintain normal and/or regain lost liver function, including regenerating damaged or lost hepatic tissue and/or inhibiting additional damage thereto. These compounds
- 15 are referred to herein as morphogen-stimulating agents, and are understood to include substances which, when administered to a mammal, act on cells of tissue(s) or organ(s) that normally are responsible for, or capable of, producing a morphogen and/or secreting a morphogen,
- 20 and which cause the endogenous level of the morphogen to be altered. The agent may act, for example, by stimulating expression and/or secretion of an endogenous morphogen.
- 25 While the methods and compositions described herein are particularly related to liver organ therapies, as will be appreciated by those skilled in the art, the methods and compositions of this invention can be applied, without undue experimentation, to other organ
- 30 applications, including but not limited to, the pancreas, lung, kidney and heart. Accordingly, the methods and compositions disclosed herein can be used to advantage in the repair, regeneration, transplantation and/or function level enhancement of
- 35 damaged or lost tissue such as, for example, damaged

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lung tissue resulting from emphysema, cirrhotic kidney or pancreatic tissue, damaged heart or blood vessel tissue, as may result from cardiomyopathies and/or atherothrombotic or cardioembolic strokes, damaged
5 stomach tissue resulting from ulceric perforations or their repair, damaged neural tissue as may result from physical injury, degenerative diseases such as Alzheimer's disease or multiple sclerosis or strokes, and damaged dental and/or periodontal tissue as may
10 result from disease or mechanical injury. The methods and compositions also may be used to protect these tissues from anticipated injury, including unavoidably or deliberately induced injury, as may occur in a surgical or other clinical procedure. In addition to
15 the tissue regenerative properties provided herein, the gene therapy and drug delivery protocols described herein may be used to particular advantage in pancreatic tissue, renal tissue and lung tissue contexts.

20

As embodied herein, the expression "maintaining normal liver function" means both regaining or restoring liver function lost due to a hepatocellular injury or inborn metabolic defect, as well as
25 protecting the hepatic tissue at risk of damage from hepatocellular injury. "Depressed liver function" level refers to a diminished or deficient liver function as a result of a tissue injury or disease. The expression "enhance viability of" transplant
30 hepatic tissue or organ, as used herein, means protection from, reduction of and/or elimination of reduced or lost tissue or organ function as a result of tissue necrosis and/or fibrosis associated with transplantation, particularly immune response-mediated
35 tissue necrosis and/or fibrosis. "Alleviating" means

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- protection from, reduction of and/or elimination of undesired tissue destruction, particularly immune response-mediated tissue destruction. "Transplanted" living tissue includes both tissue grafts and cellular
- 5 transplants, as in the case of transplanted isolated progenitor or stem cells, for example, which may be implanted alone or in association with a temporary scaffolding. Tissues may be autologous or allogenic tissue and/or synthetic tissue created, for example, by
- 10 culturing hepatic cells in the presence of an artificial matrix. "Morphogenically permissive environment" is understood to mean an environment competent to allow tissue morphogenesis to occur. Finally, "symptom alleviating cofactor" refers to one
- 15 or more pharmaceuticals which may be administered together with the therapeutic agents of this invention and which alleviate or mitigate one or more of the symptoms typically associated with liver tissue and/or liver function loss. Exemplary cofactors include
- 20 antibiotics, antiseptics, non-steroidal anti-inflammatory agents, and the like.

- In one aspect of the invention, the methods and compositions of this invention are useful in the
- 25 replacement of diseased, damaged or lost hepatic tissue in a mammal, particularly when the damaged tissue interferes with normal tissue or organ function. Where hepatic tissue has been lost, remaining hepatocytes are capable only of compensatory cell division to return
- 30 the organ volume essentially to its original size. As determined by extensive experimental partial hepatectomy studies wherein part of all of a liver lobe is excised, this compensatory growth does not involve true morphogenesis, and the lost tissue is not itself

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regenerated. Rather, the intact lobe is capable only of tissue augmentation to compensate for the lost mass. By contrast, recent studies on toxin-induced tissue damage does suggest that this repair involves

- 5 morphogenesis, particularly the infiltration and proliferation of progenitor cells. As described in Example 3 and 4, below, endogenous morphogen expression is enhanced following toxin-induced hepatic tissue damage, and not following partial hepatectomy.

10

- When the proteins described herein are provided to, or their expression stimulated at, a hepatic tissue locus, the developmental cascade of tissue morphogenesis is induced, capable of stimulating the
- 15 migration, proliferation and differentiation of hepatic progenitor cells, to regenerate viable hepatic tissue, including inducing the necessary associated vascularization (see below). Thus, in one embodiment the invention provides methods and compositions for
- 20 regenerating lost or substantially irreparably damaged hepatic tissue. The morphogen preferably is provided directly to the locus of tissue regeneration, e.g., by injection of the morphogen dispersed in a biocompatible, injectable solution, or by topical
- 25 administration, as by painting or spraying a morphogen-containing solution on the tissue. Preferably, the locus has been surgically prepared by removing existing necrotic or cirrhotic tissue. Alternatively, morphogen may be provided locally by means of an osmotic pump
- 30 implanted in the peritoneal cavity. At least one morphogen (OP-1) is known to be expressed by hepatic tissue during liver formation. Accordingly, in the alternative, and/or in addition, an agent capable of stimulating expression and/or secretion of an
- 35 endogenous morphogen may be administered. As yet

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another alternative, progenitor hepatocytic cells may be stimulated ex vivo by exposure to a morphogen or morphogen-stimulating agent, and the stimulated cells, now primed for proliferation and differentiation, then
5 provided to the hepatic tissue locus. A morphogen or a morphogen-stimulating agent also may be implanted with the cells. Alternatively, a suitable local morphogen concentration may be maintained by means, for example, of an osmotic pump. In all these cases the existing
10 tissue provides the necessary matrix requirements, providing a suitable substratum for the proliferating and differentiating cells in a morphogenically permissive environment, as well as providing the necessary signals for directing the tissue-specificity
15 of the developing tissue.

When the morphogens (or progenitor cells stimulated by these morphogens) are provided at a tissue-specific locus (e.g., by systemic injection or by implantation
20 or injection at a tissue-specific locus, or by administration of an agent capable of stimulating morphogen expression in vivo), the existing tissue at that locus, whether diseased or damaged, has the capacity of acting as a suitable matrix.
25 Alternatively, a formulated matrix may be externally provided together with the stimulated progenitor cells or morphogen, as may be necessary when the extent of injury sustained by the damaged tissue is large. The matrix should be a biocompatible, suitably modified
30 acellular matrix having dimensions such that it allows the influx, differentiation, and proliferation of migratory progenitor cells, and is capable of providing a morphogenically permissive environment (see *infra*).

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Currently preferred matrices also are biodegradable. Where morphogen and/or progenitor cells are to be implanted and the existing liver tissue is insufficient to provide the necessary matrix components, the
5 formulated matrix preferably is tissue-specific.

Formulated matrices may be generated from a fibrin clot or dehydrated organ-specific tissue, prepared for example, by treating the tissue with solvents to
10 substantially remove the non-structural components from the tissue. Alternatively, the matrix may be formulated synthetically using one or more biocompatible, preferably in vivo biodegradable, structural carrier materials such as collagen, laminin,
15 and/or hyaluronic acid which also may be in association with suitable tissue-specific cell attachment factors. Other biocompatible, in vivo biodegradable components, including synthetic polymers, including polybutyric, polylactic, polyglycolic acids, polyanhydrides and/or
20 copolymers thereof. Currently preferred structural materials contain collagens. Currently preferred cell attachment factors include glycosaminoglycans and proteoglycans. The matrix further may be treated with
25 an agent or agents to increase the number of pores and/or micropits on its surfaces, so as to enhance the influx, proliferation and differentiation of migratory progenitor cells from the body of the mammal.

In many instances, the loss of hepatic tissue
30 function results from fibrosis or scar tissue formation, formed in response to an initial or repeated injury to the tissue. The degree of scar tissue formation generally depends on the regenerative properties of the injured tissue, and on the degree and
35 type of injury. In liver, repeated tissue damage

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results in liver cirrhosis which destroys normal hepatic architecture by fiborous septa, causing vascular disorganization and perfusion deficits that impair liver function and unchecked, lead to hepatic failure. Thus, in another aspect, the invention provides methods and compositions that may be used to prevent and/or substantially inhibit the formation of scar tissue in hepatic tissue by providing the morphogens, or morphogen-stimulated cells, to a newly injured tissue locus (see below).

The morphogens of this invention also may be used to increase or regenerate a liver progenitor or stem cell population in a mammal. For example, progenitor cells may be isolated from an individual's bone marrow, stimulated ex vivo for a time and at a morphogen concentration sufficient to induce the cells to proliferate, and returned to the bone marrow. Other sources of progenitor cells that may be suitable include biocompatible cells obtained from a cultured cell line, stimulated in culture, and subsequently provided to the body. Alternatively, the morphogen may be provided systemically, or implanted, injected or otherwise provided to a progenitor cell population in an individual to induce its mitogenic activity in vivo. For example, an agent capable of stimulating morphogen expression in the progenitor cell population of interest may be provided to the cells in vivo, for example systemically, to induce mitogenic activity.

In still another aspect of the invention, the morphogens also may be used to support the growth and maintenance of differentiated cells, inducing existing differentiated cells to continue expressing their phenotype. It is anticipated that this activity will

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be particularly useful in the treatment of liver disorders where loss of liver function is caused by cells becoming metabolically senescent or quiescent. Application of the protein directly to the cells to be
5 treated, or providing it by systemic injection, can be used to stimulate these cells to continue expressing their phenotype, thereby significantly reversing the effects of the dysfunction. Alternatively, administration of an agent capable of stimulating
10 morphogen expression in vivo also may be used. In addition, the morphogens of this invention also may be used in gene therapy protocols to stimulate the growth of quiescent cells, thereby potentially enhancing the ability of these cells to incorporate exogenous DNA.

15

In another aspect of the invention, the method disclosed is useful for redifferentiating transformed cells, particularly transformed cells of parenchymal origin, such that the morphogen-treated cells are
20 induced to display a morphology characteristic of untransformed cells. As described in international application US92/01968 (WO92/15323) and [CRP070PC] the morphogens previously have been found to induce redifferentiation of transformed embryonic cells and
25 cells of neuronal origin to a morphology characteristic of untransformed cells. Morphogen treatment preferably induces cell rounding and cell aggregation (clumping), cell-cell adhesion, and CAM production. The methods described herein are anticipated to substantially
30 inhibit or reduce hepatocytic cell tumor formation and/or proliferation in liver tissue. It is anticipated that the methods of this invention will be useful in substantially reducing the effects of various carcinomas and sarcomas of liver tissue origin,
35 including hepatocellular carcinomas, bileduct

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carcinomas, hepatoblastomas, and hemangiosarcomas. In addition, the method also is anticipated to aid in inhibiting neoplastic lesions caused by metastatic tissue. Metastatic tumors are one of the most common
5 neoplasms of the liver, as they can reaching the liver through the bloodstream or lymph nodes. Metastatic tumors may damage hepatic function for example, by distorting normal liver tissue architecture, blocking or inhibiting blood flow, and/or by stimulating the
10 body's immune response.

In another aspect of the invention, the morphogens described herein are useful for providing
15 hepatocellular protective effects to alleviate liver tissue damage associated with the body's immune/inflammatory response to an initial injury to the tissue. As described in detail in international application US92/07358 (WO93/04692), such a response may follow acute or chronic trauma to hepatic tissue,
20 caused, for example, by an autoimmune dysfunction, neoplastic lesion, infection, chemical or mechanical trauma, disease or by partial or complete interruption of blood flow to hepatocytes, for example following ischemia or hypoxia, or by other trauma to the liver or
25 surrounding material. For example, portal hypertension is a significant liver disease caused by reduced blood flow through the portal vein and is characterized by tissue necrosis and cirrhosis. Application of the morphogen directly to the cells to be treated, or
30 providing the morphogen to the mammal systemically, for example, intravenously or indirectly by oral administration, may be used to alleviate and/or inhibit the immunologically related response to a hepatic tissue injury. Alternatively, administration of an
35 agent capable of stimulating morphogen expression

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and/or secretion in vivo, preferably at the site of injury, also may be used. Where the injury is to be unavoidably or deliberately induced, as during surgery or other aggressive clinical treatment, the morphogen
5 or agent may be provided prior to induction of the injury to provide a cytoprotective effect to the liver tissue at risk.

Similarly, hepatic tissues and organs for
10 transplantation also are subject to the tissue destructive effects associated with the recipient host body's inflammatory response following transplantation. It is currently believed that the initial destructive response is due in large part to reperfusion injury to
15 the transplanted organ after it has been transplanted to the organ recipient.

Accordingly, the success of liver or hepatic tissue transplantation depends greatly on the preservation of
20 the tissue activity (e.g., tissue or organ viability) at the harvest of the organ, during storage of the harvested organ, and at transplantation. To date, preservation of organs such as lungs, pancreas, heart and liver remains a significant stumbling block to the
25 successful transplantation of these organs. U.S. Patent No. 4,952,409 describes a superoxide dismutase-containing liposome to inhibit reperfusion injury. U.S. Patent No. 5,002,965 describes the use of ginkgolides, known platelet activating factor
30 antagonists, to inhibit reperfusion injury. Both of these factors are described as working primarily by inhibiting the release of and/or inhibiting the damaging effects of free oxygen radicals. A number of patents also have issued on the use of
35 immunosuppressants for inhibiting graft rejection. A

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representative listing includes U.S. Patent Nos. 5,104,858, 5,008,246 and 5,068,323. A significant problem with many immunosuppressants is their low therapeutic index, requiring the administration of high
5 doses that can have significant toxic side effects.

Thus, in another aspect of the invention, where a partial or complete organ transplant is desired, the morphogen may be administered to transplant tissue to
10 enhance the viability of the tissue, to alleviate the tissue damage associated with immune response-mediated tissue destruction and/or to provide a cytoprotective effect to tissue at risk for such damage. Exemplary transplant tissues include hepatic tissue grafts which
15 may be allogenic, autologous and/or synthetic (e.g., cultured cells attached to an artificial matrix), and whole or partial livers. Where the transplant tissue (e.g., liver, lung, kidney, pancreas, heart, etc.) is to be harvested from a donor host, the morphogen also
20 preferably is provided to the tissue prior to, or concomitant with the tissue harvest, e.g., as a prophylactic, to provide a cytoprotective effect to the tissue.

25 In another aspect of the invention, the morphogens described herein also may be used in a gene therapy protocol and/or as part of a drug delivery protocol to correct a protein deficiency in a mammal, resulting, for example, from a genetic disorder or other
30 dysfunction to the protein-producing tissue. Specifically, the methods and compositions of this invention are contemplated for use in providing to the mammal an in vivo protein-producing mechanism for correcting any protein deficiency in the mammal. These
35 proteins include proteins normally expressed and/or

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secreted by hepatic tissue and which play a role in liver-related functions, proteins normally expressed and secreted by the liver and which function elsewhere in the body, and proteins not normally expressed by hepatic tissue. Cells competent for expressing one or more proteins necessary to overcome the protein deficiency in vivo may be stimulated to proliferate ex vivo, and then implanted at a morphogenically permissive site at a liver-specific tissue locus in vivo. The competent cells may be attached to a scaffold-like structure prior to implantation. Alternatively, the competent cells may be attached to a synthetic or formulated matrix and implanted together with a morphogen at an extra-hepatic site in vivo, such as within the folds of the mesentery, or other associated vascularized tissue locus capable of providing the necessary nutrients and gas exchange to the cells. A detailed description of useful extra-hepatic loci are described, for example, in WO90/12604, published November 1, 1990 to Vacanti et al., the disclosure of which is incorporated herein by reference. Exposing primary hepatocytes to a morphogen stimulates their proliferation (see below), thereby enhancing their cellular viability upon implantation, accelerating tissue development, and reducing the original cell population required to seed the matrix. In addition, implantation with a morphogen eliminates the need for partial hepatectomy to stimulate proliferation, and enhances cellular viability by inhibiting the inflammatory/immune response typically associated with such a procedure, overcoming the significant hepatocyte cell loss typically seen in this procedure.

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Cells competent for correcting a protein deficiency include allogenic primary hepatocytes, preferably from a serotypically compatible individual and competent for expressing the protein or proteins of interest, and
5 autologous cells transfected with the genetic material necessary to express the protein of interest. For example, primary hepatocytes may be removed from the patient by biopsy, transfected using standard recombinant DNA technology, proliferated, attached to a
10 matrix and reimplanted together with a morphogen. Preferably the morphogen is provided to the cells during transfection and proliferation to enhance the mitogenic activity (and nucleic acid uptake) of these cells. In a currently preferred embodiment, morphogen
15 is adsorbed to the matrix surface to which the cells are attached and the complex implanted as a single entity ("cell-matrix structure".)

In any treatment method of the invention,
20 "administration of morphogen" refers to the administration of the morphogen, either alone or in combination with other molecules. For example, the mature form of the morphogen may be provided in association with its precursor "pro" domain, which is
25 known to enhance the solubility of the protein. Alternatively, the pro form of the morphogen (e.g., defined, for example, by residues 30-431 of OP1, Seq. I.D. No. 16, see below) may be used. Other useful molecules known to enhance protein solubility include
30 casein and other milk components, as well as various serum proteins. Additional useful molecules which may be associated with the morphogen or morphogen-stimulating agent include tissue targeting molecules capable of directing the morphogen or morphogen-
35 stimulating agent to hepatic tissue. Tissue targeting

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molecules envisioned to be useful in the treatment protocols of this invention include antibodies, antibody fragments or other binding proteins which interact specifically with surface molecules on nerve tissue cells. Still another useful tissue targeting molecule may include part or all of the morphogen precursor "pro" domain.

Associated tissue targeting or solubility-enhancing molecules also may be covalently linked to the morphogen using standard chemical means, including acid-labile linkages, which likely will be preferentially cleaved in the acidic environment of bone remodeling sites.

The morphogens and morphogen-stimulating agents also may be provided to the liver tissue together with other molecules ("cofactors") known to have a beneficial effect in treating damaged hepatic tissue, particularly cofactors capable of mitigating or alleviating symptoms typically associated with hepatic tissue damage and/or loss. Examples of such cofactors include antiseptics, antibiotics, tetracycline, aminoglycosides, macrolides, penicillins and cephalosporins, and other, non-steroidal anti-inflammatory agents.

Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins, such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), all of which are presented in Table II and Seq. ID Nos.5-14), and the

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recently identified 60A protein (from Drosophila, Seq. ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218.) The members of this family, which include members of the TGF- β super-family of proteins, share substantial amino acid sequence homology in their C-terminal regions. The proteins are translated as a precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The "pro" form of the protein, includes both the pro domain and the mature domain, and forms a soluble species that appears to be the primary form secreted from cultured mammalian cells. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne ((1986) Nucleic Acids Research 14:4683-4691.) Table I, below, describes the various morphogens identified to date, including their nomenclature as used herein, their Seq. ID references, and publication sources for the amino acid sequences for the full length proteins not included in the Seq. Listing. The disclosure of these publications is incorporated herein by reference.

TABLE I

25

"OP-1" Refers generically to the group of morphogenetically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The

- 23 -

conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.) The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

15 "OP-2" refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 7 and 8. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 20 and 21 (hOP2) and Seq. ID Nos. 22 and 23 (mOP2.) The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins likely are defined essentially by residues 18-263 (hOP2) and residues 18-260

- 24 -

(mOP2). (Another cleavage site also occurs 21 residues upstream for both OP-2 proteins.)

- 5 "CBMP2" refers generically to the morphogenically active proteins expressed from a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", Seq ID No. 9) or human CBMP2B DNA ("CBMP2B(fx)", Seq. ID No. 10). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408.
- 10
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- 20
- 25 "DPP(fx)" refers to protein sequences encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Padgett, et al (1987) Nature 325: 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.
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"Vgl(fx)" refers to protein sequences encoded by the *Xenopus Vgl* gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in Weeks (1987) Cell 51: 861-867. The prodomain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.

"Vgr-1(fx)" refers to protein sequences encoded by the murine *Vgr-1* gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13). The amino acid sequence for the full length protein appears in Lyons, et al, (1989) PNAS 86: 4554-4558. The prodomain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.

"GDF-1(fx)" refers to protein sequences encoded by the human *GDF-1* gene and defining the conserved seven cysteine skeleton (Seq. ID No. 14). The cDNA and encoded amino sequence for the full length protein is provided in Seq. ID. No. 32. The prodomain likely extends from the signal peptide clavage site to residue 214; the mature protein likely is defined by residues 215-372.

"60A" refers generically to the morphogenically active proteins expressed from part or all of a DNA sequence (from the *Drosophila* 60A gene) encoding the 60A proteins (see Seq.

- 26 -

- 5 ID No. 24 wherein the cDNA and encoded amino acid sequence for the full length protein is provided). "60A(fx)" refers to the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of Seq. ID No. 24.) The prodomain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455.
- 10 "BMP3(fx)" refers to protein sequences encoded by the human BMP3 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 26). The amino acid sequence for the full length protein appears in Wozney et al. (1988) Science 242: 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.
- 15 "BMP5(fx)" refers to protein sequences encoded by the human BMP5 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 27). The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87: 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.
- 20 "BMP6(fx)" refers to protein sequences encoded by the human BMP6 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 28). The amino acid sequence for the full
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length protein appears in Celeste, et al. (1990) PNAS 87: 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.

The OP-2 proteins have an additional cysteine residue in this region (e.g., see residue 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of Seq. ID No. 14) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention. Thus, as defined herein, a morphogen is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain comprises at least the C-terminal six cysteine skeleton defined by residues 43-139 of Seq. ID No. 5, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- or inter-chain disulfide bonds such that the protein is capable of

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acting as a morphogen as defined herein. Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. In addition, it is also anticipated that these morphogens are capable of inducing redifferentiation of committed cells under appropriate environmental conditions.

In one preferred aspect, the morphogens of this invention comprise one of two species of generic amino acid sequences: Generic Sequence 1 (Seq. ID No. 1) or Generic Sequence 2 (Seq. ID No. 2); where each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof. Generic Sequence 1 comprises the conserved six cysteine skeleton and Generic Sequence 2 comprises the conserved six cysteine skeleton plus the additional cysteine identified in OP-2 (see residue 36, Seq. ID No. 2). In another preferred aspect, these sequences further comprise the following additional sequence at their N-terminus:

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)

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Preferred amino acid sequences within the foregoing generic sequences include: Generic Sequence 3 (Seq. ID No. 3), Generic Sequence 4 (Seq. ID No. 4), Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31), listed below. These Generic

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Sequences accommodate the homologies shared among the various preferred members of this morphogen family identified in Table II, as well as the amino acid sequence variation among them. Specifically, Generic Sequences 3 and 4 are composite amino acid sequences of the following proteins presented in Table II and identified in Seq. ID Nos. 5-14: human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14.) The generic sequences include both the amino acid identity shared by the sequences in Table II, as well as alternative residues for the variable positions within the sequence. Note that these generic sequences allow for an additional cysteine at position 41 or 46 in Generic Sequences 3 or 4, respectively, providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids which influence the tertiary structure of the proteins.

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Generic Sequence 3

Leu Tyr Val Xaa Phe

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Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

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Xaa Ala Pro Xaa Gly Xaa Xaa Ala

15

20

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa

- 30 -

- 25 30
 Xaa Pro Xaa Xaa Xaa Xaa Xaa
- 35
 Xaa Xaa Xaa Asn His Ala Xaa Xaa
- 5 40 45
 Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
- 50
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
- 55 60
 10 Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
- 65
 Xaa Xaa Xaa Leu Xaa Xaa Xaa
- 70 75
 Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
- 15 80
 Xaa Xaa Xaa Xaa Met Xaa Val Xaa
- 85 90
 Xaa Cys Gly Cys Xaa
- 95
- 20 wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn); Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu

- 31 -

or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at
res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 =
(Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro
or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at
5 res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala
or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala);
Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at
res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn
or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at
10 res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or
Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 =
(Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at
res.49 = (Val or Met); Xaa at res.50 = (His or Asn);
Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa
15 at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53
= (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser);
Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56
= (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at
res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or
20 Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 =
(Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at
res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg
or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at
res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro
25 or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at
res.71 = (Ser or Ala); Xaa at res.72 = (Val or Met);
Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr
or Leu); Xaa at res.76 = (Asp or Asn); Xaa at res.77 =
(Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn
30 or Tyr); Xaa at res.79 = (Ser, Asn, Asp or Glu); Xaa at
res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile or
Val); Xaa at res.84 = (Lys or Arg); Xaa at res.85 =
(Lys, Asn, Gln or His); Xaa at res.86 = (Tyr or His);

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Xaa at res.87 = (Arg, Gln or Glu); Xaa at res.88 =
 (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr or Ala);
 Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at
 res.93 = (Ala, Gly or Glu); and Xaa at res.97 = (His or
 5 Arg);

Generic Sequence 4

	Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe	
10	1	5 10
	Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa	
	15	
	Xaa Ala Pro Xaa Gly Xaa Xaa Ala	
	20	25
15	Xaa Tyr Cys Xaa Gly Xaa Cys Xaa	
	30	35
	Xaa Pro Xaa Xaa Xaa Xaa Xaa	
	40	
	Xaa Xaa Xaa Asn His Ala Xaa Xaa	
20	45	50
	Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa	
	55	
	Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys	
	60	65
25	Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa	
	70	
	Xaa Xaa Xaa Leu Xaa Xaa Xaa	
	75	80
	Xaa Xaa Xaa Xaa Val Xaa Leu Xaa	
30	85	
	Xaa Xaa Xaa Xaa Met Xaa Val Xaa	
	90	95
	Xaa Cys Gly Cys Xaa	
	100	
35		

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wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4 = (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 = (Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg, or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp or Gln); Xaa at res.33 = Glu, Lys, Asp or Gln); Xaa at res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 = (Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 = (Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His or Asn); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 = (Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp);

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Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr or Leu); Xaa at res.81 = (Asp or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser, Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa at res.102 = (His or Arg).

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Similarly, Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31) accommodate the homologies shared among all the morphogen protein family members identified in Table II. Specifically, Generic Sequences 5 and 6 are composite amino acid sequences of human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14), human BMP3 (Seq. ID No. 26), human BMP5 (Seq. ID No. 27), human BMP6 (Seq. ID No. 28) and 60(A) (from Drosophila, Seq. ID Nos. 24-25). The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine skeletons (Generic Sequences 5 and 6, respectively), as well as alternative residues for the variable positions within the sequence. As for Generic

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Sequences 3 and 4, Generic Sequences 5 and 6 allow for an additional cysteine at position 41 (Generic Sequence 5) or position 46 (Generic Sequence 6), providing an appropriate cysteine skeleton where inter- or
 5 intramolecular disulfide bonds can form, and containing certain critical amino acids which influence the tertiary structure of the proteins.

Generic Sequence 5

```

10          Leu Xaa Xaa Xaa Phe
              1             5
          Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                  10
15      Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
              15             20
          Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
                  25             30
          Xaa Pro Xaa Xaa Xaa Xaa Xaa
              35
20      Xaa Xaa Xaa Asn His Ala Xaa Xaa
              40             45
          Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                  50
25      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
              55             60
          Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
                  65
          Xaa Xaa Xaa Leu Xaa Xaa Xaa
  
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70

75

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

80

Xaa Xaa Xaa Xaa Met Xaa Val Xaa

5

85

90

Xaa Cys Xaa Cys Xaa

95

- wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.2 = (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.7 = (Asp, Glu or Lys); Xaa at res.8 = (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or Glu); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 = (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.28 = (Glu, Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro, Gln or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser, Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at

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res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at
 5 res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro or
 10 Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at
 15 res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Met or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or Leu); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 =
 20 (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln, His or Val); Xaa at res.86 = (Tyr or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn,
 25 Glu or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly or Ala) and Xaa at res.97 = (His or Arg).

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Generic Sequence 6

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe
 1 5 10
 Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
 35 15
 Xaa Xaa Pro Xaa Xaa Xaa Ala

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      20                      25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
      30                      35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
5      40
Xaa Xaa Xaa Asn His Ala Xaa Xaa
      45                      50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      55
10    Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
      60                      65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
      70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
15    75                      80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
      85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
      90                      95
20    Xaa Cys Xaa Cys Xaa
      100

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wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the

25 following: "Res." means "residue" and Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); Xaa at res.5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr); Xaa at res.7 = (Tyr or Lys); Xaa at res.8 = (Val or Ile); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.12 = (Asp, Glu, or Lys); Xaa at res.13 = (Leu, Val or Ile); Xaa at res.16 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.17 = (Asp, Arg, Asn or Glu); Xaa at res.19 = (Ile or Val);

35 Xaa at res.20 = (Ile or Val); Xaa at res.21 = (Ala or

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Ser); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg);
Xaa at res.24 = (Gly or Ser); Xaa at res.25 = (Tyr or
Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Gln,
Leu, or Gly); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at
5 res.31 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at
res.33 = Glu, Lys, Asp, Gln or Ala); Xaa at res.35 =
(Ala, Ser, Pro, Gln or Asn); Xaa at res.36 = (Phe, Leu
or Tyr); Xaa at res.38 = (Leu, Val or Met); Xaa at
res.39 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.40 =
10 (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.41 = (Tyr,
Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly
or Leu); Xaa at res.43 = (Asn, Ser or Lys); Xaa at
res.44 = (Ala, Ser, Gly or Pro); Xaa at res.45 = (Thr,
Leu or Ser); Xaa at res.49 = (Ile, Val or Thr); Xaa at
15 res.50 = (Val, Leu or Ile); Xaa at res.51 = (Gln or
Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.53
= (Leu or Ile); Xaa at res.54 = (Val or Met); Xaa at
res.55 = (His, Asn or Arg); Xaa at res.56 = (Phe, Leu,
Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn,
20 Ala, Val or Leu); Xaa at res.58 = (Asn, Lys, Ala, Glu,
Gly or Phe); Xaa at res.59 = (Pro, Ser or Val); Xaa at
res.60 = (Glu, Asp, Gly, Val or Lys); Xaa at res.61 =
(Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His);
Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro
25 or Asp); Xaa at res.64 = (Lys, Leu or Glu); Xaa at
res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val);
Xaa at res.70 = (Thr, Ala or Glu); Xaa at res.71 =
(Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or
Val); Xaa at res.73 = (Asn, Ser, Asp or Gly); Xaa at
30 res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr,
Val or Leu); Xaa at res.76 = (Ser, Ala or Pro); Xaa at
res.77 = (Val, Met or Ile); Xaa at res.79 = (Tyr or
Phe); Xaa at res.80 = (Phe, Tyr, Leu or His); Xaa at
res.81 = (Asp, Asn or Leu); Xaa at res.82 = (Asp, Glu,
35 Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn, Tyr or

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Asp); Xaa at res.84 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile, Val or Asn); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln, His or Val); Xaa at res.91 =
5 (Tyr or His); Xaa at res.92 = (Arg, Gln, Glu or Pro); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr, Ala or Ile); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly, Glu or Ser); Xaa at res.100 = (Gly or Ala); and Xaa at res.102 =
10 (His or Arg).

Particularly useful sequences for use as morphogens in this invention include the C-terminal domains, e.g., the C-terminal 96-102 amino acid residues of Vgl,
15 Vgr-1, DPP, OP-1, OP-2, CBMP-2A, CBMP-2B, GDF-1 (see Table II, below, and Seq. ID Nos. 5-14), as well as proteins comprising the C-terminal domains of 60A, BMP3, BMP5 and BMP6 (see Seq. ID Nos. 24-28), all of which include at least the conserved six or seven
20 cysteine skeleton. In addition, biosynthetic constructs designed from the generic sequences, such as COP-1, 3-5, 7, 16, disclosed in U.S. Pat. No. 5,011,691, also are useful. Other sequences include the inhibins/activin proteins (see, for example, U.S.
25 Pat. Nos. 4,968,590 and 5,011,691). Accordingly, other useful sequences are those sharing at least 70% amino acid sequence homology or "similarity", and preferably 80% homology or similarity with any of the sequences above. These are anticipated to include allelic,
30 species variants and other sequence variants (e.g., including "muteins" or "mutant proteins"), whether naturally-occurring or biosynthetically produced, as well as novel members of this morphogenic family of proteins. As used herein, "amino acid sequence
35 homology" is understood to mean amino acid sequence

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- similarity, and homologous sequences share identical or similar amino acids, where similar amino acids are conserved amino acids as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol.5, Suppl.3, pp.345-362 (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington D.C. 1978.) Thus, a candidate sequence sharing 70% amino acid homology with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 70% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence, or constitute a conserved amino acid change thereto. "Amino acid sequence identity" is understood to require identical amino acids between two aligned sequences. Thus, a candidate sequence sharing 60% amino acid identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 60% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence.

- As used herein, all homologies and identities calculated use OP-1 as the reference sequence. Also as used herein, sequences are aligned for homology and identity calculations using the method of Needleman et al. (1970) J.Mol. Biol. 48:443-453 and identities calculated by the Align program (DNASTAR, Inc.) In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are ignored when making the homology/identity calculation.

- The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most

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preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein. Accordingly, in another preferred aspect of the invention, useful morphogens
5 include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various identified species of OP1 and OP2 (Seq. ID No. 29).

10

In still another preferred aspect of the invention, useful morphogens include dimeric proteins comprising amino acid sequences encoded by nucleic acids that hybridize to DNA or RNA sequences encoding the C-
15 terminal sequences defining the conserved seven cysteine domain of OP1 or OP2, e.g., nucleotides 1036-1341 and nucleotides 1390-1695 of Seq. ID No. 16 and 20, respectively, under stringent hybridization conditions. As used herein, stringent hybridization
20 conditions are defined as hybridization in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

25 The morphogens useful in the methods, composition and devices of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA or other
30 synthetic techniques, and includes allelic and species variants of these proteins, naturally-occurring or biosynthetic mutants thereof, as well as various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including
35 those which may alter the conserved C-terminal cysteine

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skeleton, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the specifically described constructs disclosed herein.
5 The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated, chimeric and/or mutated
10 forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

The morphogenic proteins can be expressed from intact, chimeric and/or truncated cDNA or from
15 synthetic DNAs in procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently preferred host cells include E. coli or mammalian cells, such as CHO, COS or BSC cells. A detailed
20 description of the morphogens useful in the methods, compositions and devices of this invention is disclosed in copending US patent application Serial Nos. 752,764, filed August 30, 1991, and 667,274, filed March 11, 1991, the disclosure of which are incorporated herein
25 by reference.

Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or genomic libraries of various different species which encode
30 appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both procaryotes and eucaryotes, to produce large quantities of active proteins capable of maintaining liver function in a
35 mammal, including correcting liver function

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deficiencies and stimulating hepatic tissue regeneration and repair in a variety of mammals, including humans.

- 5 The foregoing and other objects, features and advantages of the present invention will be made more apparent from the following detailed description of the invention.

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Brief Description of the Drawings:

The foregoing and other objects and features of this invention, as well as the invention itself, may be more fully understood from the following description, when read together with the accompanying drawings, in which:

FIGURE 1 is a representation of a Northern blot identifying OP-1-specific mRNA expression in developing liver tissue in embryonic and postnatal mouse, wherein lanes 2 and 3 contained RNA from 15- and 20-day embryo tissue, respectively; lanes 4-8, RNA from 3, 7, 14, 21 and 28 days post natal animals, respectively; and lanes 1 and 9 were molecular weight marker ladders;

FIGURE 2 is a photomicrograph showing the effect of phosphate buffered saline (PBS, animal 1) or morphogen (OP-1, animal 2) on partially hepatectomized rats (arrow indicates the treated lobe in both animals);

FIGURE 3 is a representation of a Northern blot of mRNA isolated from sham-operated (lanes 3, 5, 7, 9, 11, 13 and 15) and partially hepatectomized rats (lanes 2, 4, 6, 8, 10, 12, 14) at 6 hr intervals between 12-96 hours post surgery, probed with an mOP-1-specific probe, and lanes 1 and 16 are molecular weight marker lanes;

FIGURE 4 is a representation of a Northern blot of mRNA isolated from galactosamine-treated rats and probed with mOP-1-specific probe on days 0-7, 10 (lanes

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1-9, respectively, and lane 10 contains molecular weight markers);

FIGURE 5 (A and B) are schematic representations of morphogen inhibition of early mononuclear phagocytic cell multinuclearization in vivo; and

FIGURE 6 (A-D) graphs the effects of a morphogen (e.g., OP-1, Figs. 6A and 6C) and TGF-B (Fig. 6B and 6D) on collagen (6A and 6B) and hyaluronic acid (6C and 6D) production in primary fibroblast cultures.

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Detailed Description of the Invention

It now has been discovered that the proteins described herein are effective agents for maintaining
5 liver function in a mammal. As described herein, these proteins ("morphogens") are capable of inducing hepatic tissue regeneration and repair under conditions where true tissue morphogenesis typically does not occur, including stimulating the proliferation and
10 differentiation of hepatocytic progenitor cells. The proteins also are capable of providing a cytoprotective effect to alleviate the tissue destructive effects associated with immunologically-related hepatic tissue damage. Accordingly, the proteins may be used as part
15 of a protocol for regenerating damaged or lost hepatic tissue, correcting a liver function deficiency, and enhancing the viability of a tissue or organ to be transplanted in a mammal. The morphogens also may be used in a gene therapy protocol to correct a protein
20 deficiency in a mammal.

Provided below are detailed descriptions of suitable morphogens useful in the methods, compositions and devices of this invention, as well as methods for
25 their administration and application, and numerous, nonlimiting examples which 1) illustrate the suitability of the morphogens and morphogen-stimulating agents described herein as therapeutic agents for maintaining liver function in a mammal; and 2) provide
30 assays with which to test candidate morphogens and morphogen-stimulating agents for their efficacy. Specifically, the examples demonstrate the expression distribution of endogenous morphogen (Example 1), the expression of endogenous morphogen during liver
35 formation in a developing embryo (Example 2), the

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ability of morphogens to induce proliferation of primary hepatocytes (Example 3), morphogen-induced liver tissue morphogenesis following partial hepatectomy (Example 4); endogenous morphogen
5 expression during hepatic tissue repair following toxin-induced tissue damage (Examples 5); the inhibitory effect of morphogens on the body's cellular and humoral immune response (Example 6); effect of morphogen on fibrogenesis (Example 7); morphogen
10 utility in liver diagnostic procedures (Example 8), and a screening assay for testing candidate morphogen-stimulating agents (Example 9).

15 I. Useful Morphogens

As defined herein a protein is morphogenic if it is capable of inducing the developmental cascade of cellular and molecular events that culminate in the
20 formation of new, organ-specific tissue and comprises at least the conserved C-terminal six cysteine skeleton or its functional equivalent (see supra). Specifically, the morphogens generally are capable of all of the following biological functions in a
25 morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells.
30 Details of how the morphogens useful in the method of this invention first were identified, as well as a description on how to make, use and test them for morphogenic activity are disclosed in USSN 667,274, filed March 11, 1991 and USSN 752,764, filed August 30,
35 1991, the disclosures of which are hereby incorporated

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by reference. As disclosed therein, the morphogens may be purified from naturally-sourced material or recombinantly produced from procaryotic or eucaryotic host cells, using the genetic sequences disclosed
5 therein. Alternatively, novel morphogenic sequences may be identified following the procedures disclosed therein.

Particularly useful proteins include those which
10 comprise the naturally derived sequences disclosed in Table II. Other useful sequences include biosynthetic constructs such as those disclosed in U.S. Pat. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5,
15 COP-7, and COP-16).

Accordingly, the morphogens useful in the methods and compositions of this invention also may be described by morphogenically active proteins having
20 amino acid sequences sharing 70% or, preferably, 80% homology (similarity) with any of the sequences described above, where "homology" is as defined herein above.

25 The morphogens useful in the method of this invention also can be described by any of the 6 generic sequences described herein (Generic Sequences 1, 2, 3, 4, 5 and 6). Generic sequences 1 and 2 also may include, at their N-terminus, the sequence

30

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)

1

5

Table II, set forth below, compares the amino acid
35 sequences of the active regions of native proteins that have been identified as morphogens, including human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1

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(Seq. ID Nos. 7, 8, and 20-23), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), BMP3 (Seq. ID No. 26), DPP (from *Drosophila*, Seq. ID No. 11), Vgl, (from *Xenopus*, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13),

5 GDF-1 (from mouse, Seq. ID Nos. 14, 32 and 33), 60A protein (from *Drosophila*, Seq. ID Nos. 24 and 25), BMP5 (Seq. ID No. 27) and BMP6 (Seq. ID No. 28). The sequences are aligned essentially following the method of Needleman et al. (1970) J. Mol. Biol., 48:443-453,

10 calculated using the Align Program (DNASTar, Inc.) In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of

15 illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser

20 and Ile.

TABLE II

25	hOP-1	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
	mOP-1
	hOP-2	...	Arg	Arg
	mOP-2	...	Arg	Arg
	DPP	...	Arg	Arg	...	Ser
30	Vgl	Lys	Arg	His

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	Vgr-1	Gly	
	CBMP-2A	Arg	...	Pro	
	CBMP-2B	...	Arg	Arg	...	Ser	
	BMP3	...	Ala	Arg	Arg	Tyr	...	Lys	...	
5	GDF-1	...	Arg	Ala	Arg	Arg	
	60A	...	Gln	Met	Glu	Thr	
	BMP5	
	BMP6	...	Arg	
		1					5			
10										
	hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
	mOP-1
	hOP-2	Gln	Leu	...
15	mOP-2	Ser	Leu	...
	DPP	Asp	...	Ser	...	Val	Asp	...
	Vgl	Glu	...	Lys	...	Val	Asn
	Vgr-1	Gln	...	Val
	CBMP-2A	Asp	...	Ser	...	Val	Asn	...
20	CBMP-2B	Asp	...	Ser	...	Val	Asn	...
	BMP3	Asp	...	Ala	...	Ile	Ser	Glu
	GDF-1	Glu	Val	His	Arg
	60A	Asp	...	Lys	His	...
	BMP5
25	BMP6	Gln
			10					15		
	hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
	mOP-1
30	hOP-2	...	Val	Gln	Ser
	mOP-2	...	Val	Gln	Ser
	DPP	Val	Leu	Asp
	Vgl	...	Val	Gln	Met
	Vgr-1	Lys
35	CBMP-2A	Val	Pro	His

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	CBMP-2B	Val	Pro	Gln
	BMP3	Ser	...	Lys	Ser	Phe	Asp
	GDF-1	...	Val	Arg	...	Phe	Leu
	60A	Gly
5	BMP5
	BMP6	Lys
				20					25	
10	hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	mOP-1
	hOP-2	Ser
	mOP-2
	DPP	His	...	Lys	...	Pro
15	Vgl	...	Asn	Tyr	Pro
	Vgr-1	...	Asn	Asp	Ser
	CBMP-2A	...	Phe	His	...	Glu	...	Pro
	CBMP-2B	...	Phe	His	...	Asp	...	Pro
	BMP3	Ser	...	Ala	...	Gln
20	GDF-1	...	Asn	Gln	...	Gln
	60A	...	Phe	Ser	Asn
	BMP5	...	Phe	Asp	Ser
	BMP6	...	Asn	Asp	Ser
				30						35
25	hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
	mOP-1
	hOP-2	Asp	...	Cys
	mOP-2	Asp	...	Cys
30	DPP	Ala	Asp	His	Phe	...	Ser
	Vgl	Tyr	Thr	Glu	Ile	Leu	...	Gly
	Vgr-1	Ala	His
	CBMP-2A	Ala	Asp	His	Leu	...	Ser
	CBMP-2B	Ala	Asp	His	Leu	...	Ser
35	GDF-1	Leu	...	Val	Ala	Leu	Ser	Gly	Ser**	...

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	BMP3	Met	Pro	Lys	Ser	Leu	Lys	Pro
	60A	Ala	His
	BMP5	Ala	His	Met
	BMP6	Ala	His	Met
5						40				
	hOP-1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
	mOP-1
	hOP-2	Leu	...	Ser	...
10	mOP-2	Leu	...	Ser	...
	DPP	Val
	Vgl	Ser	Leu
	Vgr-1
	CBMP-2A
15	CBMP-2B
	BMP3	Ser	Thr	Ile	...	Ser	Ile
	GDF-1	Leu	Val	Leu	Arg	Ala	...
	60A
	BMP5
20	BMP6
		45					50			
	hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
25	mOP-1	Asp
	hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
	mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
	DPP	...	Asn	Asn	Asn	Gly	Lys	...
	Vgl	Ser	...	Glu	Asp	Ile
30	Vgr-1	Val	Met	Tyr	...
	CBMP-2A	...	Asn	Ser	Val	...	Ser	---	Lys	Ile
	CBMP-2B	...	Asn	Ser	Val	...	Ser	---	Ser	Ile
	BMP3	...	Arg	Ala**	Gly	Val	Val	Pro	Gly	Ile
	GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
35	60A	Leu	Leu	Glu	...	Lys	Lys	...

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	hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
	mOP-1
	hOP-2	...	Ser	...	Asn	Arg
5	mOP-2	...	Ser	...	Asn	Arg
	DPP	Asn	...	Gln	...	Thr	...	Val
	Vgl	...	Asn	Asn	Asp	Val	...	Arg
	Vgr-1	Asn
	CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val
10	CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val
	BMP3	...	Glu	Asn	Lys	Val
	GDF-1	...	Asn	...	Asp	Val	...	Arg
	60A	Leu	Asn	Asp	Glu	Asn
	BMP5
15	BMP6	Asn

85

	hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg
20	mOP-1
	hOP-2	...	His	Lys
	mOP-2	...	His	Lys
	DPP	Asn	...	Gln	Glu	...	Thr	...	Val
	Vgl	His	...	Glu	Ala	...	Asp
25	Vgr-1
	CBMP-2A	Asn	...	Gln	Asp	Glu
	CBMP-2B	Asn	...	Gln	Glu	Glu
	BMP3	Val	...	Pro	Thr	...	Glu
	GDF-1	Gln	...	Glu	Asp	Asp
30	60A	Ile	...	Lys
	BMP5
	BMP6	Trp

90

95

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	hOP-1	Ala	Cys	Gly	Cys	His
	mOP-1
	hOP-2
	mOP-2
5	DPP	Gly	Arg
	Vgl	Glu	Arg
	Vgr-1
	CBMP-2A	Gly	Arg
	CBMP-2B	Gly	Arg
10	BMP3	Ser	...	Ala	...	Arg
	GDF-1	Glu	Arg
	60A	Ser
	BMP5	Ser
	BMP6
15				100		
	**Between residues 56 and 57 of BMP3 is a Val residue;					
	between residues 43 and 44 of GDF-1 lies					
	the amino acid sequence Gly-Gly-Pro-Pro.					

20

As is apparent from the foregoing amino acid sequence comparisons, significant amino acid changes can be made within the generic sequences while retaining the morphogenic activity. For example, while

25 the GDF-1 protein sequence depicted in Table II shares only about 50% amino acid identity with the hOP1 sequence described therein, the GDF-1 sequence shares greater than 70% amino acid sequence homology (or "similarity") with the hOP1 sequence, where "homology"

30 or "similarity" includes allowed conservative amino acid changes within the sequence as defined by Dayoff, et al., Atlas of Protein Sequence and Structure vol.5, supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed. Res. Fd'n, Washington D.C. 1979.)

35

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The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence

5 defining the conserved six cysteine skeleton of HOPl (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the

10 *Drosophila* 60A protein. Accordingly, in still another preferred aspect, the invention includes morphogens comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine skeleton and accommodates the identities between the various

15 identified mouse and human OP1 and OP2 proteins. OPX is presented in Seq. ID No. 29. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human

20 OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos. 16-23).

II. Matrix Considerations

25 The morphogens of this invention may be implanted surgically, dispersed in a biocompatible, preferably in vivo biodegradable matrix appropriately modified to provide a structure in which the morphogen may be dispersed and which allows the influx, differentiation

30 and proliferation of migrating progenitor cells. Alternatively, or, in addition, differentiated hepatocytes and/or hepatocytic progenitor cells, stimulated by exposure to the morphogen, may be disposed in and attached to a matrix structure and

35 implanted surgically. In certain applications, such as

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where tissue morphogenesis is to be induced in the absence of endogenous tissue-specificity directing signals, the matrix preferably also provides signals capable of directing the tissue specificity of the
5 differentiating cells, and provides a morphogenically permissive environment, being essentially free of growth inhibiting signals.

Where the matrix is to be incorporated into a
10 surgically prepared liver, or provided to a biocompatible, associated site, the formulated matrix on which the morphogen is disposed may be shaped as desired in anticipation of surgery or may be shaped by the physician or technician during surgery. Where
15 cells are to be attached to the matrix before implantation, the matrix preferably is shaped before cells are attached thereto. The matrix preferably is biodegradable in vivo, being slowly absorbed by the body and replaced by new tissue growth, in the shape or
20 very nearly in the shape of the implant.

Details of how to make and how to use preferred matrices useful in this invention are disclosed below. In addition to these matrices, WO 88/03785, published
25 June 2, 1988, and W090/12604, published November 1, 1990, describe additional polymeric materials and matrix scaffold considerations. The disclosures of these publications are incorporated herein by reference.

30

A. Tissue-derived Matrices

Suitable biocompatible, in vivo biodegradable acellular matrices may be prepared from
35 naturally-occurring tissue. The tissue is treated with suitable agents to substantially extract the cellular, nonstructural components of the tissue. The agents

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also should be capable of extracting any growth inhibiting components associated with the tissue. The resulting material is a porous, acellular matrix, substantially depleted in nonstructurally-associated components, and preferably containing structural molecules such as collagen, laminin, hyaluronic acid, and the like.

The matrix also may be further treated with agents that modify the matrix, increasing the number of pores and micropits on its surfaces. Those skilled in the art will know how to determine which agents are best suited to the extraction of nonstructural components for different tissues. For example, soft tissues such as liver and lung may be thin-sectioned and exposed to a nonpolar solvent such as, for example, 100% ethanol, to destroy the cellular structure of the tissue and extract nonstructural components. The material then is dried and pulverized to yield nonadherent porous particles. Structural tissues such as cartilage and dentin where collagen is the primary component may be demineralized and extracted with guanidine, essentially following the method of Sampath et al. (1983) PNAS 80:6591-6595. For example, pulverized and demineralized dentin is extracted with five volumes of 4M guanidine-HCl, 50mM Tris-HCl, pH 7.0 for 16 hours at 4°C. The suspension then is filtered. The insoluble material that remains is collected and used to fabricate the matrix. The material is mostly collagenous in manner. It is devoid of morphogenic activity. The matrix particles may further be treated with a collagen fibril-modifying agent that extracts potentially unwanted components from the matrix, and

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alters the surface structure of the matrix material. Useful agents include acids, organic solvents or heated aqueous media. A detailed description of these matrix treatments are disclosed in U.S. Patent No. 4,975,526 and PCT publication US90/00912, published September 7, 1990 (WO90/10018).

The currently most preferred agent is a heated aqueous fibril-modifying medium such as water, to increase the matrix particle surface area and porosity. The currently most preferred aqueous medium is an acidic aqueous medium having a pH of less than about 4.5, e.g., within the range of about pH 2 - pH 4 which may help to "swell" the collagen before heating. 0.1% acetic acid, which has a pH of about 3, currently is most preferred. 0.1 M acetic acid also may be used.

Various amounts of delipidated, demineralized guanidine-extracted collagen matrix are heated in the aqueous medium (1g matrix/30ml aqueous medium) under constant stirring in a water jacketed glass flask, and maintained at a given temperature for a predetermined period of time. Preferred treatment times are about one hour, although exposure times of between about 0.5 to two hours appear acceptable. The temperature employed is held constant at a temperature within the range of about 37°C to 65°C. The currently preferred heat treatment temperature is within the range of about 45°C to 60°C.

30

After the heat treatment, the matrix is filtered, washed, lyophilized and used for implant. Where an acidic aqueous medium is used, the matrix also is preferably neutralized prior to washing and lyophilization. A currently preferred neutralization

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buffer is a 200mM sodium phosphate buffer, pH 7.0. To neutralize the matrix, the matrix preferably first is allowed to cool following thermal treatment, the acidic aqueous medium (e.g., 0.1% acetic acid) then is removed
5 and replaced with the neutralization buffer and the matrix agitated for about 30 minutes. The neutralization buffer then may be removed and the matrix washed and lyophilized.

10 Other useful fibril-modifying treatments include acid treatments (e.g., trifluoroacetic acid and hydrogen fluoride) and solvent treatments such as dichloromethane, acetonitrile, isopropanol and chloroform, as well as particular acid/solvent
15 combinations.

After contact with the fibril-modifying agent, the treated matrix may be washed to remove any extracted components, following a form of the procedure set forth
20 below:

1. Suspend matrix preparation in TBS (Tris-buffered saline) 1g/200 ml and stir at 4°C for 2 hrs; or in 6 M urea, 50 mM Tris-HCl, 500 mM NaCl, pH 7.0
25 (UTBS) or water and stir at room temperature (RT) for 30 minutes (sufficient time to neutralize the pH);

2. Centrifuge and repeat wash step; and

30 3. Centrifuge; discard supernatant; water wash residue; and then lyophilize.

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B. Synthetic Matrices

Suitable matrix scaffolds may be created from biocompatible, preferably in vivo biodegradable synthetic polymers, including polylactic acid, polyglycolic acid, polyanhydride, polybutyric acid, and copolymers thereof, and/or synthetic-inorganic materials, such as hydroxyapatite, tricalcium phosphate, and other calcium phosphates. These polymers are well described in the art and are available commercially. For example, polymers composed of polyactic acid (e.g., MW 100 kDa), 80% polylactide/20% glycoside or poly 3-hydroxybutyric acid (e.g., MW 30 kDa) all may be purchased from PolySciences, Inc. The polymer compositions generally are obtained in particulate form and the osteogenic devices preferably fabricated under nonaqueous conditions (e.g., in an ethanol-trifluoroacetic acid solution, EtOH/TFA) to avoid hydrolysis of the polymers. In addition, one can alter the morphology of the particulate polymer compositions, for example to increase porosity, using any of a number of particular solvent treatments known in the art.

For example, osteogenic devices fabricated with morphogenic protein, solubilized in EtOH/TFA as described below, and a matrix composed of polylactic acid, poly 3-hydroxybutyric acid, or 80% polylactide/20% glycoside are all osteogenically active when implanted in the rat model and bioassayed as described in U.S. Pat. No. 4,968,590 (e.g., as determined by calcium content, alkaline phosphatase levels and histology of 12-day implants).

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C. Synthetic Tissue-Specific Matrices

In addition to the naturally-derived tissue-specific matrices described above, useful
5 tissue-specific matrices may be formulated synthetically if appropriately modified. These porous biocompatible, in vivo biodegradable synthetic matrices are disclosed in PCT publication US91/03603, published December 12, 1991 (WO91/18558), the disclosure of which
10 is hereby incorporated by reference. Briefly, the matrix comprises a porous crosslinked structural polymer of biocompatible, biodegradable collagen and appropriate, tissue-specific glycosaminoglycans as tissue-specific cell attachment factors. Collagen
15 derived from a number of sources may be suitable for use in these synthetic matrices, including insoluble collagen, acid-soluble collagen, collagen soluble in neutral or basic aqueous solutions, as well as those collagens which are commercially available.

20 Glycosaminoglycans (GAGs) or mucopolysaccharides are hexosamine-containing polysaccharides of animal origin that have a tissue specific distribution, and therefore may be used to help determine the tissue
25 specificity of the morphogen-stimulated differentiating cells. Reaction with the GAGs also provides collagen with another valuable property, i.e., inability to provoke an immune reaction (foreign body reaction) from an animal host.

30 Chemically, GAGs are made up of residues of hexoseamines glycosidically bound and alternating in a more-or-less regular manner with either hexouronic acid or hexose moieties (see, e.g., Dodgson et al. in
35 Carbohydrate Metabolism and its Disorders (Dickens et al., eds.) Vol. 1, Academic Press (1968)). Useful GAGs

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include hyaluronic acid, heparin, heparin sulfate, chondroitin 6-sulfate, chondroitin 4-sulfate, dermatan sulfate, and keratin sulfate. Other GAGs are suitable for forming the matrix described herein, and those skilled in the art will either know or be able to ascertain other suitable GAGs using no more than routine experimentation. For a more detailed description of mucopolysaccharides, see Aspinall, Polysaccharides, Pergamon Press, Oxford (1970). For example, as disclosed in U.S. Application Serial No. 529,852, chondroitin-6-sulfate can be used where endochondral bone formation is desired. Heparin sulfate, on the other hand, may be used to formulate synthetic matrices for use in lung tissue repair.

Collagen can be reacted with a GAG in aqueous acidic solutions, preferably in diluted acetic acid solutions. By adding the GAG dropwise into the aqueous collagen dispersion, coprecipitates of tangled collagen fibrils coated with GAG results. This tangled mass of fibers then can be homogenized to form a homogeneous dispersion of fine fibers and then filtered and dried.

Insolubility of the collagen-GAG products can be raised to the desired degree by covalently cross-linking these materials, which also serves to raise the resistance to resorption of these materials. In general, any covalent cross-linking method suitable for cross-linking collagen also is suitable for cross-linking these composite materials, although crosslinking by a dehydrothermal process is preferred.

When dry, the crosslinked particles are essentially spherical, with diameters of about 500 μm . Scanning electron microscopy shows pores of about 20 μm on the surface and 40 μm on the interior. The interior is

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made up of both fibrous and sheet-like structures, providing surfaces for cell attachment. The voids interconnect, providing access to the cells throughout the interior of the particle. The material appears to
5 be roughly 99.5% void volume, making the material very efficient in terms of the potential cell mass that can be grown per gram of microcarrier.

10 D. Morphogen Adsorption to Matrix Surfaces

The morphogens described herein can be combined and dispersed in a suitable matrix using any of the methods described below:

15 1. Ethanol Precipitation

Matrix is added to the morphogen dissolved in guanidine-HCl. Samples are vortexed and incubated at a low temperature. Samples are then further vortexed.
20 Cold absolute ethanol is added to the mixture which is then stirred and incubated. After centrifugation (microfuge, high speed) the supernatant is discarded. The matrix is washed with cold concentrated ethanol in water and then lyophilized.
25

2. Acetonitrile Trifluoroacetic
Acid Lyophilization

In this procedure, morphogen in an
30 acetonitrile trifluoroacetic acid (ACN/TFA solution is added to the carrier material. Samples are vigorously vortexed many times and then lyophilized.

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3. Buffered Saline Lyophilization

Morphogen preparations in physiological saline may also be vortexed with the matrix and lyophilized to produce morphogenically active material.

III. Hepatocytic Cell Considerations

Primary hepatocytes or progenitor cells may be implanted in the mammal in one embodiment of the invention. For example, implanted hepatocytes may act as gene therapy tools capable of correcting a protein deficiency in vivo by expressing and/or secreting the deficient protein when implanted at a liver tissue or associated locus in a mammal. The liver functions in part as a protein-synthesizing organ, responsible for the production of myriad proteins which are secreted from the liver and transported, e.g., via the circulatory system, to function elsewhere in the body. Accordingly, hepatic tissue, like renal and pancreatic tissue, provides an endogenous system having the necessary mechanisms in place to act as a vector for the in vivo production of (including secretion of) any protein, including proteins not normally expressed by hepatic tissue. Thus, protein deficiencies that can be treated by this method include proteins involved in normal liver functions, proteins normally produced and secreted by the liver to function elsewhere in the body, and proteins not normally produced by hepatic tissue. Where the proteins to be produced are not normally expressed by hepatic tissue, the hepatocytes must be provided with means for expressing that protein. For example, the cell may be genetically engineered as described below to induce expression of the endogenous genetic sequence encoding the protein.

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Alternatively, a nucleic acid encoding the protein and under control of a suitable promoter (and enhancer), may be provided to the cell as described below. In addition, the cell may be provided with one or more
5 regulatory elements so that expression of the protein of interest mimics that of the endogenously produced protein, particularly where normal protein expression depends on changes in the physiological concentration of a molecule. For example, insulin production is
10 regulated by blood glucose levels in the body.

The protein deficiency to be corrected may result from defective endogenous protein production, including protein expression and/or secretion, or the protein's
15 efficacy may be reduced due to a preexisting condition in the individual. The defect may be genetic or may be induced by, for example, damage to the protein-synthesizing tissue. Exemplary hepatic proteins that may be used in a gene therapy include,
20 but are not limited to, albumin and albumin synthesis proteins, blood clotting factors, including fibrinogen and thrombin, Factor VIII, iron or copper binding proteins, and vitamin A binding proteins. Exemplary non-hepatic proteins that may be used in a gene therapy
25 include, but are not limited to, insulin, tissue plasminogen activator (TPA), erythropoietin, growth hormones, and the like. Similarly, the cells also may act as in vivo drug delivery vehicles, capable of producing and secreting one or more therapeutic drugs
30 when implanted at a suitable locus in a mammal. The cells further may be manipulated to modify antigen expression on the cell surface, and limit the in vivo immune response typically induced by foreign material.

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Where cells act as gene therapy tools, the cells may be obtained from a donor competent for providing the protein of interest. Cells can be obtained by biopsy or surgical excision from a donor, or from
5 established cell lines. Preferably, allogenic cells are obtained from a biocompatible donor. Alternatively, autologous cells may be obtained from the patient and modified by recombinant DNA technology to incorporate genetic sequences sufficient to allow
10 the cells to produce the protein or proteins of interest in vivo when the cells are reimplanted in the patient. Protocols and detailed discussions of considerations for introducing foreign genetic material into cells, particularly human cells, are well
15 described in the art. A representative, but by no means exhaustive list, includes US Pat.No. 4,868,116, issued September 19, 1989, US Pat. No. 4,980,286, issued December 25, 1990, both to Morgan et al., and US Pat. No. 4,396,601, issued August 2, 1983, to Salser et
20 al., Anderson, WF (1992) Science 256:808-813, Karson et al., (1992) J. Reprod Med 37:508-514, and Hoeg et al., (1990) Trans Assoc. Am Physicians 103:73-79, these disclosures of which are incorporated herein by
reference.

25

A currently preferred protocol for isolating primary hepatocytes from liver tissue is described in Example 3 below. Other methods known in the art also are envisioned to be useful, such as those described,
30 for example, in WO 88/03785. Where pluripotential hemopoietic stem cells are to be used, a useful method for their isolation is described in international application US92/01968 (WO92/15323). Briefly, and as described in detail therein, a biocompatible matrix
35 material able to allow the influx of migratory

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- progenitor cells may be implanted at an in vivo site long enough to allow the influx of migratory progenitor cells. For example, a bone-derived, guanidine-extracted matrix, formulated as disclosed for example
- 5 in Sampath et al. ((1983) PNAS 80:6591-6595), or U.S. Patent No. 4,975,526, may be implanted into a rat, essentially following the method of Sampath et al. (ibid). After three days the implant is removed, and the progenitor cells associated with the matrix
- 10 dispersed and cultured. Another method is described, for example, in US Pat. No. 5,061,620, issued 10/29/91, to Tsukamoto et al.

- Isolated cells may be stimulated in vitro by
- 15 morphogen exposure, essentially as described in Example 3. Stimulation is performed under sterile conditions, using an appropriate morphogen concentration and incubation period to stimulate the cells. Preferred times and concentration for a given procedure may be
- 20 determined empirically by the clinician without undue experimentation. In general, a period of from about 10 minutes to 72 hours should be sufficient. Cells may be attached to a matrix by incubating the cells in the presence of matrix for at least a number of hours,
- 25 e.g., 3-5 hours, or, preferably overnight. An efficient technique for attaching cells to a matrix surface is to place a concentrated suspension of cells on the surface of the matrix material and allow the cells to infiltrate and adsorb to the material. Cells
- 30 typically attach individually or in small groups. In the absence of added morphogen cells begin rearranging into clusters within 24 hours and within 3 days cells have almost completely infiltrated the support and have organized into large clusters.

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In a particularly preferred embodiment, the morphogen first is adsorbed to the matrix surface and cells subsequently attached thereto. The cell-matrix structure may be maintained in vitro and to allow the
5 cells to proliferate (preferably by exposure to a morphogen or morphogen-stimulating agent) or, alternatively, the complex may be implanted in the animal and the cells allowed to proliferate (and differentiate) in vivo.

10

As with morphogen administrations, where implanted cells are to replace damaged or lost tissue at a liver-specific locus, the cells preferably are provided to a surgically prepared locus where from which necrotic or
15 cirrhotic tissue has been removed, e.g., by surgical, chemical, ablating, or other means known in the medical art. The cells then are provided to the prepared site, preferably attached to a matrix and associated with a morphogen or morphogen-stimulating
20 agent.

The cells may be provided to a morphogenically permissive site in a liver-specific locus, e.g., following removal of necrotic and/or cirrhotic tissue,
25 or following excision of sufficient tissue to provide a morphogenically permissive site. Alternatively, the cell-matrix structure may be implanted together with a morphogen or morphogen-stimulating agent at a suitable, vascularized liver-associated locus, such as within the
30 folds of the mesentery.

As described above, implanting cells together with a morphogen or morphogen-stimulating agent enhances their proliferation and their viability in vivo, such
35 that the new tissue is formed without the significant

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associated cell loss or delay which characterizes existing protocols and which currently require the use of substantial initial seed cell populations. In addition, hepatic tissue growth can be stimulated using the methods described herein without the need of a partial hepatectomy as described in the art. Finally, the morphogens described herein functionally inhibit the tissue damage associated with the body's immune response, reducing the need for associated treatments with immunosuppressive drugs.

IV. Bioassay Considerations

The following sets forth various procedures for evaluating the in vivo morphogenic utility of the morphogens and morphogenic compositions of this invention. The proteins and compositions may be injected or surgically implanted in a mammal, following any of a number of procedures well known in the art.

Histological Evaluation

Histological sectioning and staining is preferred to determine the extent of morphogenesis in vivo, particularly in tissue repair procedures. Excised implants are fixed in Bouins Solution, embedded in paraffin, and cut into 6-8 μ m sections. Staining with toluidine blue or hemotoxylin/eosin demonstrates clearly the ultimate development of the new tissue. Twelve day implants are usually sufficient to determine whether the implants contain newly induced tissue.

Successful implants exhibit a controlled progression through the stages of induced tissue development allowing one to identify and follow the

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tissue-specific events that occur. For example, in endochondral bone formation the stages include:

- (1) leukocytes on day one; (2) mesenchymal cell migration and proliferation on days two and three;
- 5 (3) chondrocyte appearance on days five and six;
- (4) cartilage matrix formation on day seven;
- (5) cartilage calcification on day eight; (6) vascular invasion, appearance of osteoblasts, and formation of new bone on days nine and ten; (7) appearance of
- 10 osteoclasts and bone remodeling and dissolution of the implanted matrix on days twelve to eighteen; and
- (8) hematopoietic bone marrow differentiation in the ossicle on day twenty-one. Similarly, in hepatic
- tissue formation the stages include leukocytes on day
- 15 one, mesenchymal cell migration and proliferation on days two and three, hepatocyte appearance on days five and six, followed by matrix formation and vascularization.

20 Biological Markers

In addition to histological evaluation, biological markers may be used as a marker for tissue morphogenesis. Useful markers include tissue-specific

- 25 enzymes whose activities may be assayed (e.g., spectrophotometrically) after homogenization of the implant. These assays may be useful for quantitation and for obtaining an estimate of tissue formation quickly after the implants are removed from the animal.
- 30 For example, alkaline phosphatase activity may be used as a marker for osteogenesis.

Incorporation of systemically provided morphogens may be followed using tagged morphogens (e.g.,

- 35 radioactively labelled) and determining their localization in new tissue, and/or by monitoring their

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disappearance from the circulatory system using a standard pulse-chase labeling protocol. The morphogen also may be provided with a tissue-specific molecular tag, whose uptake may be monitored and correlated with the concentration of morphogen provided.

V. Formulations and Methods for Parenteral Administration of Therapeutic Agents

The morphogens of this invention may be used to repair diseased or damaged mammalian tissue. The tissue to be repaired is preferably assessed, and excess necrotic or interfering scar tissue removed as needed, by surgical, chemical, ablating or other methods known in the medical arts.

The morphogen then may be provided directly to the tissue locus as part of a sterile, biocompatible composition, either by surgical implantation or injection. Alternatively, a sterile, biocompatible composition containing morphogen-stimulated progenitor cells may be provided to the tissue locus. The existing tissue at the locus, whether diseased or damaged, provides the appropriate matrix to allow the proliferation and tissue-specific differentiation of progenitor cells. In addition, a damaged or diseased tissue locus, particularly one that has been further assaulted by surgical means, provides a morphogenically permissive environment. For some tissues, it is envisioned that systemic provision of the morphogen will be sufficient.

In some circumstances, particularly where tissue damage is extensive, the tissue may not be capable of providing a sufficient matrix for cell influx and proliferation. In these instances, it may be necessary to provide the morphogen or morphogen-stimulated

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progenitor cells to the tissue locus in association with a suitable, biocompatible formulated matrix, prepared by any of the means described below. The matrix preferably is tissue-specific, in vivo biodegradable, and comprises particles having dimensions within the range of 70-850 μ m, most preferably 150-420 μ m.

The morphogens may be provided to an individual by any suitable means. Preferably, the morphogen or morphogen-stimulating agent (collectively described herein below as the "therapeutic agent") is provided directly to the liver tissue (e.g., locally, as by injection to the tissue locus or by periodic release from a locally implanted osmotic pump). While not currently preferred for most liver tissue regenerative applications, oral administration or systemic injection also may be viable administration routes for certain applications, such as part of a protocol to enhance viability of a tissue to be transplanted, or as part of a protocol to maintain liver function during a surgical or other therapeutic procedure, or for maintaining liver function in aged or immuno-suppressed individuals, or others at risk for hepatic tissue damage. A detailed description of considerations for systemic administration, including oral and parenteral administration, is disclosed, for example, in copending [Atty. Docket CRP-059CP], incorporated hereinabove by reference. It should be noted that morphogenically active protein is present in milk, including mammary gland extract, colostrum and 57-day milk, and also is present in human serum, indicating that systemic and, in particular, oral administration are viable administrative routes for morphogens.

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Where the morphogen or morphogen-stimulating agent is provided by local injection, the morphogen preferably comprises part of an aqueous solution. The solution is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the morphogen thus may comprise normal physiologic saline (0.85-0.9% NaCl, 0.15M), pH 7-7.4. The aqueous solution containing the morphogen can be made, for example, by dissolving the protein in 50% ethanol containing acetonitrile in 0.1% trifluoroacetic acid (TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, to ten volumes of phosphate buffered saline (PBS), which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively. If desired, a given morphogen may be made more soluble by association with a suitable molecule. For example, the pro form of the morphogenic protein comprises a species that is soluble in physiologically buffered solutions. In fact, the endogenous protein is thought to be transported in this form. This soluble form of the protein may be obtained from the culture medium of morphogen-secreting mammalian cells. Alternatively, a soluble species may be formulated by complexing the mature dimer (or an active fragment thereof) with part or all of a pro domain. Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins also may be useful.

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- Useful solutions for parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences (Gennaro, A., ed.), Mack Pub., 1990. Formulations may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity. Biocompatible, preferably bioresorbable, polymers, including, for example, hyaluronic acid, collagen, polybutyrate, tricalcium phosphate, lactide and lactide/glycolide copolymers, may be useful excipients to control the release of the morphogen in vivo. Other potentially useful parenteral delivery systems for these morphogens include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes.

20

- In addition, while the mature forms of certain morphogens described herein typically are sparingly soluble, the morphogen form found in milk (and mammary gland extract and colostrum) is readily soluble, probably by noncovalent association of the mature, morphogenically active form with part or all of the pro domain of the intact sequence as described below, (see Section V.1) and/or by association with one or more milk components. Accordingly, the compounds provided herein also may be associated with molecules capable of enhancing their solubility in vitro or in vivo.

- The compounds provided herein also may be associated with molecules capable of targeting the morphogen or morphogen-stimulating agent to liver tissue. For example, an antibody, antibody fragment,

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or other binding protein that interacts specifically with a surface molecule on liver tissue cells, including hepatocytes or epithelial cells, may be used. Useful targeting molecules may be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

As described above, the morphogens provided herein share significant sequence homology in the C-terminal active domains. By contrast, the sequences typically diverge significantly in the sequences which define the pro domain. Accordingly, the pro domain is thought to be morphogen-specific. As described above, it is also known that the various morphogens identified to date are differentially expressed in the different tissues. Accordingly, without being limited to any given theory, it is likely that, under natural conditions in the body, selected morphogens typically act on a given tissue. Accordingly, part or all of the pro domains which have been identified associated with the active form of the morphogen in solution, may serve as targeting molecules for the morphogens described herein. For example, the pro domains may interact specifically with one or more molecules at the target tissue to direct the morphogen associated with the pro domain to that tissue. Accordingly, another useful targeting molecule for targeting morphogen to hepatic tissue may include part or all of a morphogen pro domain. As described above, morphogen species comprising the pro domain may be obtained from culture medium of morphogen-secreting cells. Alternatively, a tissue-targeting species may be formulated by complexing the mature dimer (or an active fragment thereof) with part or all of a pro domain.

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Finally, the morphogens or morphogen-stimulating agents provided herein may be administered alone or in combination with other molecules ("cofactors") known to be beneficial in maintaining liver function, particularly symptom-alleviating cofactors, such as other, non-steroidal anti-inflammatory agents, antiseptics and antibiotics.

The compounds provided herein can be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable nontoxic excipients and carriers. As noted above, such compositions may be prepared for direct, or local or systemic administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of powders, nasal drops, or aerosols.

The compositions can be formulated for administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which provide appropriate concentrations for a time sufficient to substantially eliminate or reduce the patient's pathological condition, including stimulating regeneration of damaged or lost hepatic tissue following hepatocellular injury including inhibiting additional damage thereto, to provide therapy for the liver diseases and disorders described above, and amounts effective to protect hepatic tissue in anticipation of injury to the tissue.

As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition will vary depending upon a number of factors, including the dosage of the drug to

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be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of therapeutic agent to be administered also is likely to depend on such variables as the type and extent of progression of the hepatic disorder, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound excipients, and its route of administration. In general terms, the compounds of this invention may be provided in an aqueous physiological buffer solution containing about 0.001 to 10% w/v compound for liquid administration. Typical dose ranges are from about 10 ng/kg to about 1 g/kg of body weight per day; a preferred dose range is from about 0.1 μ g/kg to 100 mg/kg of body weight per day. Optimally, the morphogen dosage given is between 0.1-100 μ g of protein per kilogram weight of the patient. No obvious morphogen induced pathological lesions are induced when mature morphogen (e.g., OP-1, 20 μ g) is administered daily to normal growing rats for 21 consecutive days. Moreover, 10 μ g systemic injections of morphogen (e.g., OP-1) injected daily for 10 days into normal newborn mice does not produce any gross abnormalities.

Where morphogens are administered systemically, in the methods of the present invention, preferably a large volume loading dose is used at the start of the treatment. The treatment then is continued with a maintenance dose. Further administration then can be determined by monitoring at intervals the levels of the morphogen in the blood.

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Where injury to hepatic tissue is induced deliberately as part of, for example, a surgical or other medical procedure, the morphogen preferably is provided just prior to, or concomitant with induction
5 of the trauma. Preferably, the morphogen is administered prophylactically in a surgical setting. Optimally, the morphogen dosage given in all cases is between 1-100 μg of protein per kilogram weight of the patient.

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As described above, as an alternative or, in addition, an effective amount of an agent capable of stimulating endogenous morphogen levels may be administered by any of the routes described above. For
15 example, an agent capable of stimulating morphogen production and/or secretion from liver tissue cells or cells at a distant which then is targeted to the liver, may be provided to a mammal, e.g., by direct administration of the morphogen to glial cells
20 associated with the nerve tissue to be treated. A method for identifying and testing agents capable of modulating the levels of endogenous morphogens in a given tissue is described generally herein in Example 9, and in detail in international application
25 US92/07359 (WO 93/05/72). Briefly, candidate compounds can be identified and tested by incubating the compound in vitro with a test tissue or cells thereof, for a time sufficient to allow the compound to affect the production, i.e., the expression and/or secretion, of a
30 morphogen produced by the cells of that tissue. Here, suitable tissue or cultured cells of a tissue preferably would comprise hepatic tissue cells.

A currently preferred detection means for
35 evaluating the level of the morphogen in culture upon exposure to the candidate compound comprises an immunoassay utilizing an antibody or other suitable

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binding protein capable of reacting specifically with a morphogen and being detected as part of a complex with the morphogen. Immunoassays may be performed using standard techniques known in the art and antibodies raised against a morphogen and specific for that morphogen. Agents capable of stimulating endogenous morphogens then may formulated into pharmaceutical preparations and administered as described herein.

10 V.A Soluble Morphogen Complexes

A currently preferred form of the morphogen useful in therapeutic formulations, having improved solubility in aqueous solutions and consisting essentially of amino acids, is a dimeric morphogenic protein comprising at least the 100 amino acid peptide sequence having the pattern of seven or more cysteine residues characteristic of the morphogen family complexed with a peptide comprising part or all of a pro region of a member of the morphogen family, or an allelic, species or other sequence variant thereof. Preferably, the dimeric morphogenic protein is complexed with two peptides. Also, the dimeric morphogenic protein preferably is noncovalently complexed with the pro region peptide or peptides. The pro region peptides also preferably comprise at least the N-terminal eighteen amino acids that define a given morphogen pro region. In a most preferred embodiment, peptides defining substantially the full length pro region are used.

Other soluble forms of morphogens include dimers of the uncleaved pro forms of these proteins, as well as "hemi-dimers" wherein one subunit of the dimer is an uncleaved pro form of the protein, and the other

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subunit comprises the mature form of the protein, including truncated forms thereof, preferably noncovalently associated with a cleaved pro domain peptide.

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As described above, useful pro domains include the full length pro regions, as well as various truncated forms hereof, particularly truncated forms cleaved at proteolytic Arg-Xaa-Xaa-Arg cleavage sites. For example, in OP-1, possible pro sequences include sequences defined by residues 30-292 (full length form); 48-292; and 158-292. Soluble OP-1 complex stability is enhanced when the pro region comprises the full length form rather than a truncated form, such as the 48-292 truncated form, in that residues 30-47 show sequence homology to the N-terminal portions of other morphogens, and are believed to have particular utility in enhancing complex stability for all morphogens. Accordingly, currently preferred pro sequences are those encoding the full length form of the pro region for a given morphogen. Other pro sequences contemplated to have utility include biosynthetic pro sequences, particularly those that incorporate a sequence derived from the N-terminal portion of one or more morphogen pro sequences.

As will be appreciated by those having ordinary skill in the art, useful sequences encoding the pro region may be obtained from genetic sequences encoding known morphogens. Alternatively, chimeric pro regions can be constructed from the sequences of one or more known morphogens. Still another option is to create a synthetic sequence variant of one or more known pro region sequences.

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In another preferred aspect, useful pro region peptides include polypeptide chains comprising an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions with a DNA or RNA sequence encoding at least the N-terminal eighteen amino acids of the pro region sequence for OP1 or OP2, e.g., nucleotides 136-192 and 152-211 of Seq. ID No. 16 and 20, respectively.

10 V.A.1 Isolation of Soluble morphogen complex from conditioned media or body fluid

Morphogens are expressed from mammalian cells as soluble complexes. Typically, however the complex is disassociated during purification, generally by exposure to denaturants often added to the purification solutions, such as detergents, alcohols, organic solvents, chaotropic agents and compounds added to reduce the pH of the solution. Provided below is a currently preferred protocol for purifying the soluble proteins from conditioned media (or, optionally, a body fluid such as serum, cerebro-spinal or peritoneal fluid), under non-denaturing conditions. The method is rapid, reproducible and yields isolated soluble morphogen complexes in substantially pure form.

Soluble morphogen complexes can be isolated from conditioned media using a simple, three step chromatographic protocol performed in the absence of denaturants. The protocol involves running the media (or body fluid) over an affinity column, followed by ion exchange and gel filtration chromatographies. The affinity column described below is a Zn-IMAC column. The present protocol has general applicability to the purification of a variety of morphogens, all of which

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- are anticipated to be isolatable using only minor modifications of the protocol described below. An alternative protocol also envisioned to have utility an immunoaffinity column, created using standard
- 5 procedures and, for example, using antibody specific for a given morphogen pro domain (complexed, for example, to a protein A-conjugated Sepharose column.) Protocols for developing immunoaffinity columns are well described in the art, (see, for example, Guide to
- 10 Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly sections VII and XI.)

- In this experiment OP-1 was expressed in mammalian
- 15 CHO (chinese hamster ovary) cells as described in the art (see, for example, international application US90/05903 (WO91/05802).) The CHO cell conditioned media containing 0.5% FBS was initially purified using Immobilized Metal-Ion Affinity Chromatography (IMAC).
- 20 The soluble OP-1 complex from conditioned media binds very selectively to the Zn-IMAC resin and a high concentration of imidazole (50 mM imidazole, pH 8.0) is required for the effective elution of the bound complex. The Zn-IMAC step separates the soluble OP-1
- 25 from the bulk of the contaminating serum proteins that elute in the flow through and 35 mM imidazole wash fractions. The Zn-IMAC purified soluble OP-1 is next applied to an S-Sepharose cation-exchange column equilibrated in 20 mM NaPO₄ (pH 7.0) with 50 mM NaCl.
- 30 This S-Sepharose step serves to further purify and concentrate the soluble OP-1 complex in preparation for the following gel filtration step. The protein was applied to a Sephacryl S-200HR column equilibrated in TBS. Using substantially the same protocol, soluble
- 35 morphogens also may be isolated from one or more body

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fluids, including serum, cerebro-spinal fluid or peritoneal fluid.

- IMAC was performed using Chelating-Sepharose
- 5 (Pharmacia) that had been charged with three column volumes of 0.2 M ZnSO_4 . The conditioned media was titrated to pH 7.0 and applied directly to the ZN-IMAC resin equilibrated in 20 mM HEPES (pH 7.0) with 500 mM NaCl. The Zn-IMAC resin was loaded with 80 mL of
- 10 starting conditioned media per mL of resin. After loading, the column was washed with equilibration buffer and most of the contaminating proteins were eluted with 35 mM imidazole (pH 7.0) in equilibration buffer. The soluble OP-1 complex then is eluted with
- 15 50 mM imidazole (pH 8.0) in 20 mM HEPES and 500 mM NaCl.

- The 50 mM imidazole eluate containing the soluble OP-1 complex was diluted with nine volumes of 20 mM
- 20 NaPO_4 (pH 7.0) and applied to an S-Sepharose (Pharmacia) column equilibrated in 20 mM NaPO_4 (pH 7.0) with 50 mM NaCl. The S-Sepharose resin was loaded with an equivalent of 800 mL of starting conditioned media per mL of resin. After loading the S-Sepharose column
- 25 was washed with equilibration buffer and eluted with 100 mM NaCl followed by 300 mM and 500 mM NaCl in 20 mM NaPO_4 (pH 7.0). The 300 mM NaCl pool was further purified using gel filtration chromatography. Fifty mls of the 300 mM NaCl eluate was applied to a 5.0 X 90
- 30 cm Sephacryl S-200HR (Pharmacia) equilibrated in Tris buffered saline (TBS), 50 mM Tris, 150 mM NaCl (pH 7.4). The column was eluted at a flow rate of 5 mL/minute collecting 10 mL fractions. The apparent molecular of the soluble OP-1 was determined by
- 35 comparison to protein molecular weight standards

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(alcohol dehydrogenase (ADH, 150 kDa), bovine serum albumin (BSA, 68 kDa), carbonic anhydrase (CA, 30 kDa) and cytochrome C (cyt C, 12.5 kDa). The purity of the S-200 column fractions was determined by separation on
5 standard 15% polyacrylamide SDS gels stained with coomassie blue. The identity of the mature OP-1 and the pro-domain was determined by N-terminal sequence analysis after separation of the mature OP-1 from the pro-domain using standard reverse phase C18 HPLC.

10

The soluble OP-1 complex elutes with an apparent molecular weight of 110 kDa. This agrees well with the predicted composition of the soluble OP-1 complex with one mature OP-1 dimer (35-36 kDa) associated with two
15 pro-domains (39 kDa each). Purity of the final complex can be verified by running the appropriate fraction in a reduced 15% polyacrylamide gel.

The complex components can be verified by running
20 the complex-containing fraction from the S-200 or S-200HR columns over a reverse phase C18 HPLC column and eluting in an acetonitrile gradient (in 0.1% TFA), using standard procedures. The complex is dissociated by this step, and the pro domain and mature species
25 elute as separate species. These separate species then can be subjected to N-terminal sequencing using standard procedures (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly pp. 602-613), and
30 the identity of the isolated 36kD, 39kDa proteins confirmed as mature morphogen and isolated, cleaved pro domain, respectively. N-terminal sequencing of the isolated pro domain from mammalian cell produced OP-1 revealed 2 forms of the pro region, the intact form
35 (beginning at residue 30 of Seq. ID No. 16) and a

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truncated form, (beginning at residue 48 of Seq. ID No. 16.) N-terminal sequencing of the polypeptide subunit of the isolated mature species reveals a range of N-termini for the mature sequence, beginning at
5 residues 293, 300, 313, 315, 316, and 318, of Seq. ID No. 16, all of which are active as demonstrated by the standard bone induction assay.

V.A.2. In Vitro Soluble Morphogen Complex Formation

10

As an alternative to purifying soluble complexes from culture media or a body fluid, soluble complexes may be formulated from purified pro domains and mature dimeric species. Successful complex formation
15 apparently requires association of the components under denaturing conditions sufficient to relax the folded structure of these molecules, without affecting disulfide bonds. Preferably, the denaturing conditions mimic the environment of an intracellular vesicle
20 sufficiently such that the cleaved pro domain has an opportunity to associate with the mature dimeric species under relaxed folding conditions. The concentration of denaturant in the solution then is decreased in a controlled, preferably step-wise manner,
25 so as to allow proper refolding of the dimer and pro regions while maintaining the association of the pro domain with the dimer. Useful denaturants include 4-6M urea or guanidine hydrochloride (GuHCl), in buffered solutions of pH 4-10, preferably pH 6-8. The soluble
30 complex then is formed by controlled dialysis or dilution into a solution having a final denaturant concentration of less than 0.1-2M urea or GuHCl, preferably 1-2 M urea of GuHCl, which then preferably can be diluted into a physiological buffer. Protein
35 purification/renaturing procedures and considerations

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are well described in the art, and details for developing a suitable renaturing protocol readily can be determined by one having ordinary skill in the art. One useful text on the subject is Guide to Protein

- 5 Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly section V. Complex formation also may be aided by addition of one or more chaperone proteins.

10 V.A.3 Stability of Soluble Morphogen Complexes

- The stability of the highly purified soluble morphogen complex in a physiological buffer, e.g., tris-buffered saline (TBS) and phosphate-buffered
- 15 saline (PBS), can be enhanced by any of a number of means. Currently preferred is by means of a pro region that comprises at least the first 18 amino acids of the pro sequence (e.g., residues 30-47 of Seq. ID NO. 16 for OP-1), and preferably is the full length pro
- 20 region. Residues 30-47 show sequence homology to the N-terminal portion of other morphogens and are believed to have particular utility in enhancing complex stability for all morphogens. Other useful means for enhancing the stability of soluble morphogen complexes
- 25 include three classes of additives. These additives include basic amino acids (e.g., L-arginine, lysine and betaine); nonionic detergents (e.g., Tween 80 or Nonidet P-120); and carrier proteins (e.g., serum albumin and casein). Useful concentrations of these
- 30 additives include 1-100 mM, preferably 10-70 mM, including 50 mM, basic amino acid; 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (v/v) nonionic detergent; and 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (w/v) carrier protein.

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VI. Examples

Example 1. Identification of Morphogen-Expressing Tissue

5

- Determining the tissue distribution of morphogens may be used to identify different morphogens expressed in a given tissue, as well as to identify new, related morphogens. Tissue distribution also may be used to
- 10 identify useful morphogen-producing tissue for use in screening and identifying candidate morphogen-stimulating agents. The morphogens (or their mRNA transcripts) readily are identified in different tissues using standard methodologies and minor
- 15 modifications thereof in tissues where expression may be low. For example, protein distribution may be determined using standard Western blot analysis or immunofluorescent techniques, and antibodies specific to the morphogen or morphogens of interest. Similarly,
- 20 the distribution of morphogen transcripts may be determined using standard Northern hybridization protocols and transcript-specific probes.

- Any probe capable of hybridizing specifically to a
- 25 transcript, and distinguishing the transcript of interest from other, related transcripts may be used. Because the morphogens described herein share such high sequence homology in their active, C-terminal domains, the tissue distribution of a specific morphogen
- 30 transcript may best be determined using a probe specific for the pro region of the immature protein and/or the N-terminal region of the mature protein. Another useful sequence is the 3' non-coding region flanking and immediately following the stop codon.
- 35 These portions of the sequence vary substantially among

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the morphogens described herein, and accordingly, are specific for each protein. For example, a particularly useful Vgr-1-specific probe sequence is the PvuII-SacI fragment, a 265 bp fragment encoding both a portion of the untranslated pro region and the N-terminus of the mature sequence (see Lyons et al. (1989) PNAS 86:4554-4558 for a description of the cDNA sequence). Similarly, particularly useful mOP-1-specific probe sequences are the BstXI-BglI fragment, a 0.68 Kb sequence that covers approximately two-thirds of the mOP-1 pro region; a StuI-StuI fragment, a 0.2 Kb sequence immediately upstream of the 7-cysteine domain; and the Earl-PstI fragment, an 0.3 Kb fragment containing a portion of the 3'untranslated sequence (See Seq. ID No. 18, where the pro region is defined essentially by residues 30-291.) Similar approaches may be used, for example, with hOP-1 (Seq. ID No. 16) or human or mouse OP-2 (Seq. ID Nos. 20 and 22.)

Using these morphogen-specific probes, which may be synthetically engineered or obtained from cloned sequences, morphogen transcripts can be identified in mammalian tissue, using standard methodologies well known to those having ordinary skill in the art. Briefly, total RNA is prepared from various adult murine tissues (e.g., liver, kidney, testis, heart, brain, thymus and stomach) by a standard methodology such as by the method of Chomczyaski et al. ((1987) Anal. Biochem 162:156-159) and described below. Poly (A)+ RNA is prepared by using oligo (dT)-cellulose chromatography (e.g., Type 7, from Pharmacia LKB Biotechnology, Inc.). Poly (A)+ RNA (generally 15 µg) from each tissue is fractionated on a 1% agarose/formaldehyde gel and transferred onto a Nytran membrane (Schleicher & Schuell). Following the

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transfer, the membrane is baked at 80°C and the RNA is cross-linked under UV light (generally 30 seconds at 1 mW/cm²). Prior to hybridization, the appropriate probe is denatured by heating. The hybridization is carried
5 out in a lucite cylinder rotating in a roller bottle apparatus at approximately 1 rev/min for approximately 15 hours at 37°C using a hybridization mix of 40% formamide, 5 x Denhardts, 5 x SSPE, and 0.1% SDS. Following hybridization, the non-specific counts are
10 washed off the filters in 0.1 x SSPE, 0.1% SDS at 50°C.

Examples demonstrating the tissue distribution of various morphogens, including Vgr-1, OP-1, BMP2, BMP3, BMP4, BMP5, GDF-1, and OP-2 in developing and adult
15 tissue are disclosed international application US92/01968 (WO92/15323), and in Ozkaynak, et al., (1991) Biochem. Biophys. Res. Commun. 179:116-123, and Ozkaynak, et al. (1992) (J. Biol. Chem. 267: 25220-25227), the disclosures of which are incorporated
20 herein by reference. Using the general probing methodology described herein, northern blot hybridizations using probes specific for these morphogens to probe brain, spleen, lung, heart, liver and kidney tissue indicate that kidney-related tissue
25 appears to be the primary expression source for OP-1, with brain, heart and lung tissues being secondary sources. Lung tissue appears to be the primary tissue expression source for Vgr-1, BMP5, BMP4 and BMP3. Lower levels of Vgr-1 also are seen in kidney and heart
30 tissue, while the liver appears to be a secondary expression source for BMP5, and the spleen appears to be a secondary expression source for BMP4. GDF-1 appears to be expressed primarily in brain tissue. To date, OP-2 appears to be expressed primarily in early
35 embryonic tissue. Specifically, northern blots of

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murine embryos and 6-day post-natal animals shows abundant OP2 expression in 8-day embryos. Expression is reduced significantly in 17-day embryos and is not detected in post-natal animals.

5

Example 2. Morphogen Localization in Developing Hepatic Tissue

The onset of liver formation in a developing embryo occurs at day 14. Using the hybridization protocol described in Example 1, morphogen expression was identified at the onset of liver formation during embryo development. Specifically, northern blots of mRNA isolated from murine embryo liver tissue (probed at 15 days and 20 days) and post natal mouse liver tissue (probed at 7, 14, 21 and 28 days past birth) show mOP-1 expression in developing liver tissue only during the time of liver formation. Specifically, as illustrated, in Fig. 1, mOP-1 RNA is expressed significantly in the 15 day embryo, and is present at much lower amounts at later times in healthy hepatic tissue. In the figure, lanes 2 and 3 contain RNA from 15- and 20-day embryo tissue, respectively; lanes 4-8, RNA from 3, 7, 14, 21 and 28 days post natal animals, respectively; and lane 9 is a molecular weight ladder. Lanes 1 and 9 are markers. In the Northern blot mOP-1 RNA appears as a discrete band running at about 4kb and 2.2 or 2.4 kb, as well as a shorter band at 1.8kb (see, for example, Ozkaynak, et al. (1991) Biochem. Biophys Res. 179: 116-123.)

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Example 3. Mitogenic Effect of Morphogen on Rat Hepatocytes

The ability of a morphogen to induce proliferation of primary hepatocytes may be demonstrated in vitro

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isolated from rat liver. Unless otherwise indicated, all chemicals referenced are standard, commercially available reagents, readily available from a number of sources, including Sigma Chemical, Co., St. Louis; Calbiochem, Corp., San Diego, and Aldrich Chemical Co., Milwaukee.

Rat primary hepatocyte cultures were prepared by a two-step collagenase digestion essentially as described by Fausto et al. (1987) Cell Separation: Methods and Selected Applications 4:45-77 the disclosure of which is incorporated herein by reference. Briefly, the liver of a male rat (e.g., CD strain, Charles River Laboratories, Wilmington, MA) was perfused via the portal vein with Ca^{2+} free and Mg^{2+} free Hank's balanced salt solution for 10 min at a flow of 30-40 ml/min, followed by perfusion with 0.05% collagenase in Ca^{2+} -containing medium (Hepes buffer) for 10 min. The liver capsule was removed, the cells shaken loose from the tissue and filtered hepatocytes were collected by repeated centrifugation of the cell suspension at 50 xg for 25 min. Hepatocyte suspensions were virtually free of non-parenchymal cell contamination. Cells (2×10^5 per dish) were plated on 35-mm dishes coated with rat tail collagen in MEM (modified Eagle's Medium, Gibco, Long Island) containing 5% fetal bovine serum (FBS), 1mM pyruvate, 0.2mM aspartate, 1mM proline, 0.2mM serine, 2mM glutamine, and 0.5 μg of hydrocortisone and 1 μg of insulin per ml. The cells were incubated for 24 hours under standard at 37°C, at which time the growth medium was replaced with serum-free MEM.

The cell culture then was divided into two groups: (1) wells which received morphogen within the dose range of 1-100 ng of morphogen per ml medium; and (2)

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- the control group, which received no additional factors. In this example, OP-1 was the morphogen tested. The cells then were incubated for an additional 18-24 hours after which the wells were
- 5 pulsed with $2\mu\text{Ci}/\text{well}$ of ^3H -thymidine and incubated for six more hours. The excess label then was washed off with a cold solution of 0.15 M NaCl. 250 μl of 10% trichloroacetic acid then was added to each well and the wells incubated at room temperature for 30 minutes.
- 10 The cells then were washed three times with cold distilled water, and lysed by the addition of 250 μl of 1% sodium dodecyl sulfate (SDS) for a period of 30 minutes at 37°C . The cell lysates then were harvested using standard means well known in the art, and the
- 15 incorporation of ^3H -thymidine into cellular DNA was determined by liquid scintillation as an indication of mitogenic activity of the cells.

- Morphogen treatment of primary hepatocyte cultures
- 20 significantly stimulates ^3H -thymidine incorporation into DNA, and thus promotes their cell proliferation. The mitogenesis stimulated by 20 ng of OP-1 in 1 ml serum-free medium was equivalent to the mitogenic effect of 10% fresh serum alone. By contrast, other
- 25 local-acting growth factors, such as TGF- β do not stimulate proliferation of primary hepatocytes (see Fausto et al. (1991) Ciba Found Symp 157:165-174.)

30 Example 4. Morphogen-Induced Liver Regeneration

While hepatocytes have a remarkable capacity to undergo compensatory growth following tissue loss, the reparative properties of liver differ significantly from embryonic morphogenesis. Specifically, following

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a partial hepatectomy wherein a liver lobe is partially or completely removed, the remaining intact lobes grow rapidly and double in weight due to the ability of the differentiated hepatocytes in the intact lobe to
5 undergo limited proliferation. However, the excised lobe itself is not regenerated. The following example demonstrates the ability of morphogens to regenerate lost hepatic tissue following a partial hepatectomy, including regenerating the excised tissue lobe. The
10 protocol described below is a variation on a standard partial hepatectomy protocol, described, for example, by Higgins et al. (1931) Arch. Pathol. 12:136-202 and Braun et al. (1989) PNAS 86:1558-1562, the disclosures of which are incorporated herein by reference.

15 Morphogen, e.g., purified recombinant human OP-1, mature form, was solubilized (1 mg/ml) in 50% ethanol (or compatible solvent) containing 0.1% trifluoroacetic acid (or compatible acid). The injectable OP-1
20 solution was prepared by diluting one volume of OP-1/solvent-acid stock solution with 9 volumes of 0.2% rat serum albumin in sterile PBS (phosphate-buffered saline).

25 Growing rats or aged rats were anesthetized by using ketamine. Two of the liver lobes (left and right) were cut out (approximately 1/3 of the lobe) and the morphogen was injected locally at multiple sites along the cut ends. The amount of OP-1 injected was
30 100 μ g in 100 of PBS/RSA (phosphate-buffered saline/rat serum albumin) injection buffer. Placebo samples were injection buffer without OP-1. Five rats in each group were used. The wound was closed using standard surgical procedures and the rats were allowed to eat
35 normal food and drink tap water.

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After 12 days, the rats were sacrificed and liver regeneration was observed visually. The photomicrograph in Fig. 2 illustrates dramatically the regenerative effects of OP-1 on liver tissue formation. In the figure, the arrow indicates the treated lobe. The OP-1-injected group showed complete liver tissue regeneration including reformation of the excised lobe tissue, and showed no sign of any cut in the liver (animal 2). By contrast, in the control group into which only PBS was injected, the excised lobe tissue was not regenerated (animal 1). The original incision remains in this sample.

In a related experiment, animals were partially hepatectomized or sham-operated and Northern blot analysis performed on RNA isolated from the liver tissue. None of the animals were morphogen-treated. As determined by Northern blot analysis (probed with mOP-1-specific labeled oligonucleotide, see Fig.3), in the absence of morphogen treatment, the level of endogenous morphogen is not enhanced significantly following partial hepatectomy. In the figure lanes 2, 4, 6, 8, 10, 12, and 14, are samples from partially hepatectomized rats and lanes 3, 5, 7, 9, 11, 13, and 15 are samples from sham-operated rats, and lanes 1 and 16 are markers. Samples were taken at 6 hour intervals between 12 and 96 hours post surgery.

Example 5. Morphogen Expression in Regenerating Liver Tissue Following Toxin-Induced Tissue Damage

Hepatic tissue repair following toxic agent-induced damaged tissue involves proliferation and

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differentiation of hepatocyte precursor cells. This tissue reparation apparently mimics the tissue morphogenesis cascade that occurs during embryogenesis (Fausto, et al.(1989) Lab.Investigation 60:4-13). As demonstrated in the example below, morphogen expression is enhanced significantly during hepatic tissue regeneration following galactosamine or carbon tetrachloride (CCl_4)-induced liver damage. Experiments were performed essentially as described in Kuhlmann et al., (1980) Virchows Arch 387:47-57, the disclosure of which is incorporated herein by reference .

In this experiment, male rats were provided with a single intraperitoneal injection of galactosamine-HCl 0.75 g/.kg body weight on day 0, and morphogen expression monitored by standard Northern blot of liver tissue samples taken on days 1-7 and day 10. OP-1 expression was significantly enhanced during this hepatic tissue regenerative period, indicating that morphogens play a significant role in tissue regeneration. A representation of the Northern blot is presented in Fig. 4. In Fig. 4, lanes 1-8 are samples taken on days 0-7; lane 9 is a sample taken on day 10, and lane 10 contains molecular weight markers. OP-1 mRNA shows a significant expression spike on days 3-7. Similar results were seen with tissue regeneration stimulated following CCl_4 -induced tissue, wherein CCl_4 intoxication is induced by orally administering 1.5g CCl_4 /kg body weight. Significant morphogen expression (mOP-1 mRNA, as determined by standard Northern blot) is identified by a hybridization spike at 12 hours and continuing through at least 72 hours.

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Example 6. Morphogen Inhibition of Cellular and
 Humoral Inflammatory Response

5 The morphogens described herein may be used to
alleviate tissue damage associated with immune
response-mediated damage to liver tissue. Details of
this damage and the use of morphogens to alleviate this
injury as well as to provide a cytoprotective effect in
anticipation of this injury for example, during a
10 transplant procedure, are disclosed in international
application US92/07358 (WO93/04672). A primary source
of such damage to hepatic tissue results, for example,
from reduced perfusion of the hepatic blood supply
and/or from partial or complete occlusion of the portal
15 vein. As described in international application
US92/07358 (WO93/04672) morphogens have been shown to
alleviate damage to myocardial tissue following
ischemia-reperfusion injury. The morphogens also
alleviate analogous tissue damage to hepatic tissue.

20 Morphogens described herein inhibit multinucleation
of mononuclear phagocytic cells under conditions where
these cells normally would be activated, e.g., in
response to a tissue injury or the presence of a
25 foreign substance. For example, in the absence of
morphogen, an implanted substrate material (e.g.,
implanted subcutaneously) composed of, for example,
mineralized bone, a ceramic such as titanium oxide or
any other substrate that provokes multinucleated giant
30 cell formation, rapidly becomes surrounded by
multinucleated giant cells, e.g., activated phagocytes
stimulated to respond and destroy the foreign object.
In the presence of morphogen however, the recruited
cells remain in their mononuclear precursor form and

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the matrix material is undisturbed. Figure 5 illustrates this effect of morphogens, in a schematic representation of histology results of a titanium oxide substrate implanted subcutaneously. In the figure, "mg" means multinucleated giant cells and "ob" means osteoblasts. The substrate represented in Fig. 5B was implanted together with morphogen (OP-1) and newly formed osteoblasts are evident surrounding the substrate. By contrast, the substrate represented in Fig. 5A was implanted without morphogen and extensive multinucleated giant cell formation is evident surrounding the substrate. Accordingly, the morphogens' effect in inhibiting excessive bone mass loss in a mammal also may include inhibiting activation of these giant cells.

In addition, the morphogens described herein also suppress antibody production stimulated in response to a foreign antigen in a mammal. Specifically, when bovine bone collagen matrix alone was implanted in a bony site in a rat, a standard antibody response to the collagen is stimulated in the rat as determined by standard anti-bovine collagen ELISA experiments performed on blood samples taken at four week intervals following implantation (e.g., between 12 and 20 weeks.) Serum anti-collagen antibody titers, measured by ELISA essentially following the procedure described by Nagler-Anderson et al, (1986) PNAS 83:7443-7446, the disclosure of which is incorporated herein by reference, increased consistently throughout the experiment. However, when the matrix was implanted together with a morphogen (e.g., OP-1, dispersed in the matrix and adsorbed thereto, essentially as described in U.S. Pat. No. 4,968,590) anti-bovine collagen

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antibody production was suppressed significantly. This ability of morphogen to suppress the humoral response is further evidence of morphogen utility in alleviating tissue damage associated with autoimmune diseases, including autoantibody diseases, such as rheumatoid arthritis.

Example 7. Morphogen Effect on Fibrogenesis and Scar Tissue Formation

10

The morphogens described herein induce tissue morphogenesis of damaged or lost tissue. The ability of these proteins to regenerate new tissue also is enhanced by the anti-inflammatory effect of these proteins. Provided below are a series of in vitro experiments demonstrating the ability of morphogens to induce migration and accumulation of mesenchymal cells. In addition, the experiments demonstrate that morphogens, unlike TGF- β , do not stimulate fibrogenesis or scar tissue formation. Specifically, morphogens do not stimulate production of collagen, hyaluronic acid (HA) or metalloproteinases in primary fibroblasts, all of which are required for fibrogenesis or scar tissue formation. By contrast, TGF- β , a known inducer of fibrosis, but not of tissue morphogenesis as described herein, does stimulate production of these fibrosis markers.

Chemotaxis and migration of mesenchymal progenitor cells were measured in modified Boyden chambers essentially as described by Fava, R.A. et al (1991) J. Exp. Med. 173: 1121-1132, the disclosure of which is incorporated herein by reference, using polycarbonate filters of 2, 3 and 8 micron pores to measure migration

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of progenitor neutrophils, monocytes and fibroblasts. Chemotaxis was measured over a range of morphogen concentrations, e.g., 10^{-20} M to 10^{-12} M OP-1. For progenitor neutrophils and monocytes, 10^{-18} - 10^{-17} M OP-1 consistently induced maximal migration, and 10^{-14} to 10^{-13} M OP-1 maximally induced migration of progenitor fibroblasts. In all cases the chemotactic activity could be inhibited with anti-OP-1 antibody. Similar migration activities also were measured and observed with TGF- β .

The effect of morphogen on fibrogenesis was determined by evaluating fibroblast production of hyaluronic acid (HA), collagen, collagenase and tissue inhibitor of metalloproteinases (TIMP).

Human fibroblasts were established from explants of infant foreskins and maintained in monolayer culture using standard culturing procedures. (See, for example, (1976) J. Exp. Med. 144: 1188-1203.) Briefly, fibroblasts were grown in maintenance medium consisting of Eagle's MEM, supplemented with nonessential amino acids, ascorbic acid (50 μ g/ml), NaHCO_3 and HEPES buffers (pH 7.2), penicillin (100 U/ml), streptomycin (100 μ g/ml), amphotericin B (1 μ g/ml) and 9% heat inactivated FCS. Fibroblasts used as target cells to measure chemotaxis were maintained in 150 mm diameter glass petri dishes. Fibroblasts used in assays to measure synthesis of collagen, hyaluronic acid, collagenase and tissue inhibitors of metalloproteinases (TIMP) were grown in 100 mm diameter plastic tissue culture petri dishes.

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The effects of morphogen on fibroblast production of hyaluronic acid, collagens, collagenase and TIMP were determined by standard assays (See, for example, Posttethwaite et al. (1989) J. Clin. Invest. 83: 629-636, Posttethwaite (1988) J. Cell Biol. 106: 311-318 and Clark et al (1985) Arch. Bio-chem Biophys. 241: 36-44, the disclosures of which are incorporated by reference.) For these assays, fibroblasts were transferred to 24-well tissue culture plates at a density of 8×10^4 cells per well. Fibroblasts were grown confluent in maintenance medium containing 9% FCS for 72 h and then grown in serum-free maintenance medium for 24 h. Medium was then removed from each well and various concentrations of OP-1 (recombinantly produced mature or soluble form) or TGF- β -1 (R&D Systems, Minneapolis) in 50 μ l PBS were added to triplicate wells containing the confluent fibroblast monolayers. For experiments that measured production of collagenase and TIMP, maintenance medium (450 μ l) containing 5% FCS was added to each well, and culture supernatants were harvested from each well 48 h later and stored at -70°C until assayed. For experiments that assessed HA production, maintenance medium (450 μ l) containing 2.5% FCS was added to each well, and cultures grown for 48 h. For experiments that measured fibroblast production of collagens, serum-free maintenance medium (450 μ l) without non-essential amino acids was added to each well and cultures grown for 72 h. Fibroblast production of HA was measured by labeling newly synthesized glycosaminoglycans (GAG) with [^3H]-acetate the last 24 h of culture and quantitating released radioactivity after incubation with hyaluronidase from Streptomyces hyalurolyticus (ICN Biochemicals, Cleveland, OH) which specifically

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degrades hyaluronic acid. Production of total collagen by fibroblasts was measured using a collagenase-sensitive protein assay that reflects [³H]-proline incorporation the last 24 h of culture into newly synthesized collagens. Collagenase and TIMP protein levels in fibroblast cultures supernatants was measured by specific ELISAs.

As shown in Fig. 6, OP1 does not stimulate significant collagen or HA production, as compared with TGF- β . In the figure, panel A shows OP-1 effect on collagen production, panel B shows TGF- β effect on collagen production, and panels C and D show OP-1 (panel C) and TGF- β (panel D) effect on HA production. The morphogen results were the same whether the soluble or mature form of OP1 was used. By contrast, the latent form of TGF- β (e.g., pro domain-associated form of TGF- β) was not active.

20 Example 8. Liver Tissue Diagnostics

Morphogen localization in developing and regenerating liver tissue can be used as part of a method for diagnosing a liver function disorder *vivo*. The method may be particularly advantageous for diagnosing early stages of a liver dysfunction associated with a hepatocellular injury. Specifically, a biopsy of liver tissue is performed on a patient at risk, using standard procedures known in the medical art. Morphogen expression associated with the biopsied tissue then is assessed using standard methodologies, as by immunolocalization, using standard immunofluorescence techniques in concert with morphogen-specific antisera or monoclonal antibodies.

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Specifically, the biopsied tissue is thin sectioned using standard methodologies known in the art, and fluorescently labelled (or otherwise detectable) antibodies having specificity for the morphogen are incubated with the tissue under conditions sufficient to allow specific antigen-antibody complex formation. The presence and quantity of complex formed then is detected and compared with a predetermined standard or reference value. Detection of altered levels of morphogen present in the tissue then may be used as an indicator of tissue dysfunction. Alternatively, fluctuation in morphogen levels may be assessed by monitoring morphogen transcription levels, either by standard Northern blot analysis or by in situ hybridization, using a labelled probe capable of hybridizing specifically to morphogen RNA and standard RNA hybridization protocols well described in the art and as described in Examples 1, 2, 5 and 6.

Fluctuations in morphogen levels present in the bloodstream or peritoneal fluid also may be used to evaluate liver tissue viability. For example, morphogens are detected associated with regenerating liver tissue and/or may be released from dying cells into surrounding peritoneal fluid. OP-1 recently has been identified in human blood, which also may be a means of morphogen transport.

Serum samples may be obtained by standard venipuncture and serum prepared by centrifugation at 3,000 RPM for ten minutes. Similarly, peritoneal fluid samples may be obtained by a standard fluid extraction methodology. The presence of morphogen in the serum or peritoneal fluid then may be assessed by standard

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Western blot (immunoblot), ELISA or RIA procedures. Briefly, for example, with the ELISA, samples may be diluted in an appropriate buffer, such as phosphate-buffered saline, and 50 μ l aliquots allowed to absorb to flat bottomed wells in microtitre plates pre-coated with morphogen-specific antibody, and allowed to incubate for 18 hours at 4°C. Plates then may be washed with a standard buffer and incubated with 50 μ l aliquots of a second morphogen-specific antibody conjugated with a detecting agent, e.g., biotin, in an appropriate buffer, for 90 minutes at room temperature. Morphogen-antibody complexes then may be detected using standard procedures.

Alternatively, a morphogen-specific affinity column may be created using, for example, morphogen-specific antibodies adsorbed to a column matrix, and passing the fluid sample through the matrix to selectively extract the morphogen of interest. The morphogen then is eluted. A suitable elution buffer may be determined empirically by determining appropriate binding and elution conditions first with a control (e.g., purified, recombinantly-produced morphogen.) Fractions then are tested for the presence of the morphogen by standard immunoblot. Morphogen concentrations in serum or other fluid samples then may be determined using standard protein quantification techniques, including by spectrophotometric absorbance or by quantitation by ELISA or RIA antibody assays. Using this procedure, OP-1 has been identified in serum.

OP-1 was detected in human serum using the following assay. A monoclonal antibody raised against mammalian, recombinantly produced OP-1 using standard

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immunology techniques well described in the art and described generally in Example 13, was immobilized by passing the antibody over an activated agarose gel (e.g., Affi-GelTM, from Bio-Rad Laboratories, Richmond, CA, prepared following manufacturer's instructions), and used to purify OP-1 from serum. Human serum then was passed over the column and eluted with 3M K-thiocyanate. K-thiocyanate fractions then were dialyzed in 6M urea, 20mM PO₄, pH 7.0, applied to a C8 HPLC column, and eluted with a 20 minute, 25-50% acetonitrile/0.1% TFA gradient. Mature, recombinantly produced OP-1 homodimers elute between 20-22 minutes. Accordingly, these fractions from the affinity-purified human serum sample were collected and tested for the presence of OP-1 by standard immunoblot using an OP-1-specific antibody, and the protein identity confirmed by N-terminal sequencing.

Morphogens may be used in diagnostic applications by comparing the quantity of morphogen present in a body fluid sample with a predetermined reference value, with fluctuations in fluid morphogen levels indicating a change in the status of liver tissue. Alternatively, fluctuations in the level of endogenous morphogen antibodies may be detected by this method, most likely in serum, using an antibody or other binding protein capable of interacting specifically with the endogenous morphogen antibody. Detected fluctuations in the levels of the endogenous antibody may be used as indicators of a change in tissue status.

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Example 9. Screening Assay for Candidate Compounds
 which Alter Endogenous Morphogen Levels

Candidate compound(s) which may be administered to
5 affect the level of a given morphogen may be found
using the following screening assay, in which the level
of morphogen production by a cell type which produces
measurable levels of the morphogen is determined with
and without incubating the cell in culture with the
10 compound, in order to assess the effects of the
compound on the cell's production of morphogen. This
can be accomplished by detection of the morphogen
either at the protein or RNA level. A more detailed
description also may be found in international
15 application US92/07359 (WO93/05172).

9.1 Growth of Cells in Culture

Cell cultures of kidney, adrenals, urinary bladder,
20 brain, or other organs, may be prepared as described
widely in the literature. For example, kidneys may be
explanted from neonatal or new born or young or adult
rodents (mouse or rat) and used in organ culture as
whole or sliced (1-4 mm) tissues. Primary tissue
25 cultures and established cell lines, also derived from
kidney, adrenals, urinary, bladder, brain, mammary, or
other tissues may be established in multiwell plates (6
well or 24 well) according to conventional cell culture
techniques, and are cultured in the absence or presence
30 of serum for a period of time (1-7 days). Cells may be
cultured, for example, in Dulbecco's Modified Eagle
medium (Gibco, Long Island, NY) containing serum (e.g.,
fetal calf serum at 1%-10%, Gibco) or in serum-deprived
medium, as desired, or in defined medium (e.g.,

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containing insulin, transferrin, glucose, albumin, or other growth factors).

Samples for testing the level of morphogen production includes culture supernatants or cell lysates, collected periodically and evaluated for OP-1 production by immunoblot analysis (Sambrook et al., eds., 1989, Molecular Cloning, Cold Spring Harbor Press, Cold Spring Harbor, NY), or a portion of the cell culture itself, collected periodically and used to prepare polyA+ RNA for mRNA analysis. To monitor de novo OP-1 synthesis, some cultures are labeled according to conventional procedures with an ^{35}S -methionine/ ^{35}S -cysteine mixture for 6-24 hours and then evaluated to OP-1 synthesis by conventional immunoprecipitation methods.

9.2 Determination of Level of Morphogenic Protein

In order to quantitate the production of a morphogenic protein by a cell type, an immunoassay may be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that protein. For example, OP-1 may be detected using a polyclonal antibody specific for OP-1 in an ELISA, as follows.

1 $\mu\text{g}/100 \mu\text{l}$ of affinity-purified polyclonal rabbit IgG specific for OP-1 is added to each well of a 96-well plate and incubated at 37°C for an hour. The wells are washed four times with 0.167M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1% Tween 20. To minimize non-specific binding, the wells are blocked by filling completely with 1% bovine serum albumin (BSA) in BSB and incubating for 1 hour at 37°C.

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- The wells are then washed four times with BSB containing 0.1% Tween 20. A 100 μ l aliquot of an appropriate dilution of each of the test samples of cell culture supernatant is added to each well in triplicate and incubated at 37°C for 30 min. After incubation, 100 μ l biotinylated rabbit anti-OP-1 serum (stock solution is about 1 mg/ml and diluted 1:400 in BSB containing 1% BSA before use) is added to each well and incubated at 37°C for 30 min. The wells are then washed four times with BSB containing 0.1% Tween 20. 100 μ l streptavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in BSB containing 0.1% Tween 20 before use) is added to each well and incubated at 37°C for 30 min. The plates are washed four times with 0.5M Tris buffered Saline (TBS), pH 7.2. 50 μ l substrate (ELISA Amplification System Kit, Life Technologies, Inc., Bethesda, MD) is added to each well and incubated at room temperature for 15 min. Then, 50 μ l amplifier (from the same amplification system kit) is added and incubated for another 15 min at room temperature. The reaction is stopped by the addition of 50 μ l 0.3 M sulphuric acid. The OD at 490 nm of the solution in each well is recorded. To quantitate OP-1 in culture media, a OP-1 standard curve is performed in parallel with the test samples.

- Polyclonal antibody may be prepared as follows. Each rabbit is given a primary immunization of 100 ug/500 μ l E. coli produced OP-1 monomer (amino acids 328-431 in SEQ ID NO:5) in 0.1% SDS mixed with 500 μ l Complete Freund's Adjuvant. The antigen is injected subcutaneously at multiple sites on the back and flanks of the animal. The rabbit is boosted after a month in

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- the same manner using incomplete Freund's Adjuvant. Test bleeds are taken from the ear vein seven days later. Two additional boosts and test bleeds are performed at monthly intervals until antibody against
- 5 OP-1 is detected in the serum using an ELISA assay. Then, the rabbit is boosted monthly with 100 μ g of antigen and bled (15 ml per bleed) at days seven and ten after boosting.
- 10 Monoclonal antibody specific for a given morphogen may be prepared as follows. A mouse is given two injections of E. coli produced OP-1 monomer. The first injection contains 100 μ g of OP-1 in complete Freund's adjuvant and is given subcutaneously. The second
- 15 injection contains 50 μ g of OP-1 in incomplete adjuvant and is given intraperitoneally. The mouse then receives a total of 230 μ g of OP-1 (amino acids 307-431 in SEQ ID NO:5) in four intraperitoneal injections at various times over an eight month period. One week
- 20 prior to fusion, the mouse is boosted intraperitoneally with 100 μ g of OP-1 (307-431) and 30 μ g of the N-terminal peptide (Ser₂₉₃-Asn₃₀₉-Cys) conjugated through the added cysteine to bovine serum albumin with SMCC crosslinking agent. This boost was repeated five days
- 25 (IP), four days (IP), three days (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to myeloma (e.g., 653) cells at a ratio of 1:1 using PEG 1500 (Boeringer Mannheim), and the cell fusion is plated and screened for OP-1-specific antibodies using
- 30 OP-1 (307-431) as antigen. The cell fusion and monoclonal screening then are according to standard procedures well described in standard texts widely available in the art.

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The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are
5 therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency
10 of the claims are therefore intended to be embraced therein.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: CREATIVE BIOMOLECULES, INC.
- (B) STREET: 45 SOUTH STREET
- (C) CITY: HOPKINTON
- (D) STATE: MA
- (E) COUNTRY: USA
- (F) POSTAL CODE (ZIP): 01748
- (G) TELEPHONE: 1-508-435-9001
- (H) TELEFAX: 1-508-435-0454
- (I) TELEX:

(ii) TITLE OF INVENTION: MORPHOGEN-INDUCED LIVER REGENERATION

(iii) NUMBER OF SEQUENCES: 33

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: CREATIVE BIOMOLECULES, INC.
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- (C) CITY: HOPKINTON
- (D) STATE: MA
- (E) COUNTRY: USA
- (F) ZIP: 01748

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: KELLEY ESQ, ROBIN D.
- (B) REGISTRATION NUMBER: 34,637
- (C) REFERENCE/DOCKET NUMBER: CRP-072

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 617/248-7477
- (B) TELEFAX: 617/248-7100

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
 (B) LOCATION: 1..97
 (D) OTHER INFORMATION: /label= GENERIC-SEQ1

/note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES
 ONE OF THE 20 NATURALLY-OCCURRING L-ISOMER, A-AMINO
 ACIDS, OR A DERIVATIVE THEREOF."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1             5             10             15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Cys Xaa Xaa
25             20             25             30
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
30             35             40             45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa
50             55             60
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35             65             70             75             80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
40             85             90             95
Xaa

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..97

(D) OTHER INFORMATION: /label= GENERIC-SEQ2

5 /note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES
ONE OF THE 20 NATURALLY OCCURRING L-ISOMER A-AMINO
ACIDS, OR A DERIVATIVE THEREOF."

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

15 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

20 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa
50 55 60

25 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
85 90 95

30 Xaa

(2) INFORMATION FOR SEQ ID NO:3:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..97

(D) OTHER INFORMATION: /label= GENERIC-SEQ3

45 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
50 AS DEFINED IN THE SPECIFICATION."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

55 Leu Tyr Val Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa Xaa Ala
1 5 10 15

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[illegible]

20 (2) INFORMATION FOR SEO ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

30

(ix) **FEATURE:**

(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /label= GENERIC-SE04

35 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
AS DEFINED IN THE SPECIFICATION."

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

	Cys	Xaa	Xaa	Xaa	Xaa	Leu	Tyr	Val	Xaa	Phe	Xaa	Xaa	Xaa	Gly	Trp	Xaa
	1				5					10					15	
45	Xaa	Trp	Xaa	Xaa	Ala	Pro	Xaa	Gly	Xaa	Xaa	Ala	Xaa	Tyr	Cys	Xaa	Gly
				20					25					30		
	Xaa	Cys	Xaa	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Asn	His	Ala
				35				40					45			
50	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
				50			55					60				
	Xaa	Cys	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa
	65				70					75					80	

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Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Met Xaa Val
 85 90 95

5 Xaa Xaa Cys Gly Cys Xaa
 100

(2) INFORMATION FOR SEQ ID NO:5:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 139 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
 (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..139
 (D) OTHER INFORMATION: /label= hOP1-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

30 Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
 1 5 10 15

Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser
 20 25 30

35 Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
 35 40 45

40 Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
 50 55 60

Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
 65 70 75 80

45 Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
 85 90 95

Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
 100 105 110

50 Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
 115 120 125

55 Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 130 135

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(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 5 (A) LENGTH: 139 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: MURIDAE
 (F) TISSUE TYPE: EMBRYO
- (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..139
 (D) OTHER INFORMATION: /label= MOP1-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

25 Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
 1 5 10 15

Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser
 20 25 30

30 Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
 35 40 45

Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
 50 55 60

35 Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
 65 70 75 80

Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
 40 85 90 95

Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
 100 105 110

45 Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
 115 120 125

Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 130 135

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 55 (A) LENGTH: 139 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single

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- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 5 (A) ORGANISM: HOMO SAPIENS
 (F) TISSUE TYPE: HIPPOCAMPUS
- (ix) FEATURE:
 10 (A) NAME/KEY: Protein
 (B) LOCATION: 1..139
 (D) OTHER INFORMATION: /label= HOP2-MATURE
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
- 15 Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu
 1 5 10 15
- 20 Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser
 20 25 30
- His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln
 35 40 45
- 25 Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
 50 55 60
- 30 Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn
 65 70 75 80
- Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
 85 90 95
- 35 Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
 100 105 110
- Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
 115 120 125
- 40 Arg Asn Met Val Val Lys Ala Cys Gly Cys His
 130 135
- (2) INFORMATION FOR SEQ ID NO:8:
- 45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 139 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 50 (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 55 (A) ORGANISM: MURIDAE
 (F) TISSUE TYPE: EMBRYO

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(ix) FEATURE:

- (A) NAME/KEY: Protein
 (B) LOCATION: 1..139
 (D) OTHER INFORMATION: /label= MOP2-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

10 Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu
 1 5 10 15
 Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser
 20 25 30
 15 Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg
 35 40 45
 20 Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
 50 55 60
 Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn
 65 70 75 80
 25 Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
 85 90 95
 Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
 100 105 110
 30 Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
 115 120 125
 35 Arg Asn Met Val Val Lys Ala Cys Gly Cys His
 130 135

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: bovinæ

(ix) FEATURE:

- (A) NAME/KEY: Protein
 (B) LOCATION: 1..101
 (D) OTHER INFORMATION: /label= CBMP-2A-FX

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

5 Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
 1 5 10 15
 Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly
 20 25 30
 10 Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
 35 40 45
 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala
 50 55 60
 15 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
 65 70 75 80
 Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu
 85 90 95
 20 Gly Cys Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:10:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 101 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 30 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein
 (vi) ORIGINAL SOURCE:
 35 (A) ORGANISM: HOMO SAPIENS
 (F) TISSUE TYPE: hippocampus
 (ix) FEATURE:
 (A) NAME/KEY: Protein
 40 (B) LOCATION: 1..101
 (D) OTHER INFORMATION: /label= CBMP-2B-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

45 Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
 1 5 10 15
 50 Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly
 20 25 30
 Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
 35 40 45
 55 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala
 50 55 60

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Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
 65 70 75 80
 5 Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu
 85 90 95
 Gly Cys Gly Cys Arg
 100
 10 (2) INFORMATION FOR SEQ ID NO:11:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 15 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein
 20 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: DROSOPHILA MELANOGASTER
 (ix) FEATURE:
 25 (A) NAME/KEY: Protein
 (B) LOCATION: 1..101
 (D) OTHER INFORMATION: /label= DPP-FX
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
 Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp
 1 5 10 15
 35 Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly
 20 25 30
 Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala
 35 40 45
 40 Val Val Gln Thr Leu Val Asn Asn Asn Pro Gly Lys Val Pro Lys
 50 55 60
 45 Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu
 65 70 75 80
 Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val
 85 90 95
 50 Val Gly Cys Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:12:

- 122 -

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: XENOPUS
- (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..102
 (D) OTHER INFORMATION: /label= VGL-FX
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
- | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Lys | Lys | Arg | His | Leu | Tyr | Val | Glu | Phe | Lys | Asp | Val | Gly | Trp | Gln |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Asn | Trp | Val | Ile | Ala | Pro | Gln | Gly | Tyr | Met | Ala | Asn | Tyr | Cys | Tyr | Gly |
| | | 20 | | | | | 25 | | | | | | 30 | | |
| Glu | Cys | Pro | Tyr | Pro | Leu | Thr | Glu | Ile | Leu | Asn | Gly | Ser | Asn | His | Ala |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Ile | Leu | Gln | Thr | Leu | Val | His | Ser | Ile | Glu | Pro | Glu | Asp | Ile | Pro | Leu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Pro | Cys | Cys | Val | Pro | Thr | Lys | Met | Ser | Pro | Ile | Ser | Met | Leu | Phe | Tyr |
| | 65 | | | | 70 | | | | | 75 | | | | 80 | |
| Asp | Asn | Asn | Asp | Asn | Val | Val | Leu | Arg | His | Tyr | Glu | Asn | Met | Ala | Val |
| | | | 85 | | | | | | 90 | | | | | 95 | |
| Asp | Glu | Cys | Gly | Cys | Arg | | | | | | | | | | |
| | | | 100 | | | | | | | | | | | | |
- (2) INFORMATION FOR SEQ ID NO:13:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: MURIDAE

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(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= VGR-1-FX

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

10 Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Val Gly Trp Gln
 1 5 10 15
 Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
 20 25 30
 15 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 35 40 45
 Ile Val Gln Thr Leu Val His Val Met Asn Pro Glu Tyr Val Pro Lys
 50 55 60
 20 Pro Cys Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val Leu Tyr Phe
 65 70 75 80
 25 Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
 85 90 95
 Arg Ala Cys Gly Cys His
 100

30 (2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 106 amino acids

(B) TYPE: amino acid

35 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

40 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

45 (A) ORGANISM: Homo sapiens

(F) TISSUE TYPE: brain

(ix) FEATURE:

(A) NAME/KEY: Protein

50 (B) LOCATION: 1..106

(D) OTHER INFORMATION: /note= "GDF-1 (fx)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

55

Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp His

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Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly
 20 25 30

5 Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala
 35 40 45

Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro Gly
 50 55 60

10 Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile Ser
 65 70 75 80

Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr Glu
 85 90 95

15 Asp Met Val Val Asp Glu Cys Gly Cys Arg
 100 105

20 (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 25 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys Xaa Xaa Xaa Xaa
 35 1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1822 base pairs
 40 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

50

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
 (F) TISSUE TYPE: HIPPOCAMPUS

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(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 49..1341
 (C) IDENTIFICATION METHOD: experimental
 5 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "OP1"
 /evidence= EXPERIMENTAL
 /standard_name= "OP1"

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

	GGTGGCGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG	57
		Met His Val
15		1
	CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA	105
	Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala	
	5 10 15	
20	CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC	153
	Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn	
	20 25 30 35	
25	GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG	201
	Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg	
	40 45 50	
30	CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC	249
	Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg	
	55 60 65	
35	CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG	297
	Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met	
	70 75 80	
	CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC	345
	Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly	
	85 90 95	
40	GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC	393
	Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly	
	100 105 110 115	
45	CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC	441
	Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp	
	120 125 130	
50	ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC	489
	Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe	
	135 140 145	
	CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC	537
	His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile	
55		150 155 160

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	CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC	585
	Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp	
	165 170 175	
5	TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT	633
	Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr	
	180 185 190 195	
10	CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC	681
	Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu	
	200 205 210	
15	GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC	729
	Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp	
	215 220 225	
20	ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG	777
	Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu	
	230 235 240	
25	GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC	825
	Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro	
	245 250 255	
30	AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC	873
	Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro	
	260 265 270 275	
35	TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTG CAC TTC CGC AGC ATC	921
	Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile	
	280 285 290	
40	CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC	969
	Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro	
	295 300 305	
45	AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC	1017
	Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser	
	310 315 320	
50	AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC	1065
	Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe	
	325 330 335	
55	CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC	1113
	Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala	
	340 345 350 355	
60	GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG	1161
	Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met	
	360 365 370	
65	AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC	1209
	Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn	
	375 380 385	

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CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC 1257
 Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala
 390 395 400

5 ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA 1305
 Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys
 405 410 415

10 TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC 1351
 Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG 1411
 15 GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG 1471
 TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC 1531

20 ATCCAATGAA CAAGATCTTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC 1591
 GCATAAGAAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT 1651
 CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCAGGCCA CCCAGCCGTG GGAGGAAGGG 1711
 25 GGCCTGGCAA GGGGTGGGCA CATTGGTGTG TGTGCGAAAG GAAAATTGAC CCGGAAGTTC 1771
 CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A 1822

30

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 431 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 1 5 10 15

45 Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
 20 25 30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
 35 40 45

50 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
 55 60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
 65 70 75 80

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Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
85 90 95

5 Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
100 105 110

Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
115 120 125

10 Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
130 135 140

Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
15 145 150 155 160

Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
165 170 175

20 Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
180 185 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
195 200 205

25 Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
210 215 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
30 225 230 235 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
245 250 255

35 Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
260 265 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
275 280 285

40 Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
290 295 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
45 305 310 315 320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
325 330 335

50 Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
340 345 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
355 360 365

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Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
 370 375 380

5 Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
 385 390 395 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
 405 410 415

10 Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:18:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1873 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

25

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
 (F) TISSUE TYPE: EMBRYO

30

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 104..1393
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "MOP1"
 /note= "MOP1 (CDNA)"

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

40

CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG 60

CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC 115

45

Met His Val Arg
1

TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT 163

Ser Leu Arg Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro
5 10 15 20

50

CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG 211

Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu
25 30 35

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	GTG	CAC	TCC	AGC	TTC	ATC	CAC	CGG	CGC	CTC	CGC	AGC	CAG	GAG	CGG	CGG	259
	Val	His	Ser	Ser	Phe	Ile	His	Arg	Arg	Leu	Arg	Ser	Gln	Glu	Arg	Arg	
				40					45					50			
5	GAG	ATG	CAG	CGG	GAG	ATC	CTG	TCC	ATC	TTA	GGG	TTG	CCC	CAT	CGC	CCG	307
	Glu	Met	Gln	Arg	Glu	Ile	Leu	Ser	Ile	Leu	Gly	Leu	Pro	His	Arg	Pro	
			55					60					65				
10	CGC	CCG	CAC	CTC	CAG	GGA	AAG	CAT	AAT	TCG	GCG	CCC	ATG	TTC	ATG	TTG	355
	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	Phe	Met	Leu	
			70				75						80				
15	GAC	CTG	TAC	AAC	GCC	ATG	GCG	GTG	GAG	GAG	AGC	GGG	CCG	GAC	GGA	CAG	403
	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Ser	Gly	Pro	Asp	Gly	Gln	
	85					90					95					100	
20	GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	CCC	CCT	451
	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	Pro	Pro	
					105					110					115		
	TTA	GCC	AGC	CTG	CAG	GAC	AGC	CAT	TTC	CTC	ACT	GAC	GCC	GAC	ATG	GTC	499
	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	Met	Val	
					120					125				130			
25	ATG	AGC	TTC	GTC	AAC	CTA	GTG	GAA	CAT	GAC	AAA	GAA	TTC	TTC	CAC	CCT	547
	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	His	Pro	
				135				140					145				
30	CGA	TAC	CAC	CAT	CGG	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	CCC	GAG	595
	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	Pro	Glu	
						150		155					160				
35	GGC	GAA	CGG	GTG	ACC	GCA	GCC	GAA	TTC	AGG	ATC	TAT	AAG	GAC	TAC	ATC	643
	Gly	Glu	Arg	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	Tyr	Ile	
	165					170					175					180	
40	CGG	GAG	CGA	TTT	GAC	AAC	GAG	ACC	TTC	CAG	ATC	ACA	GTC	TAT	CAG	GTG	691
	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Gln	Ile	Thr	Val	Tyr	Gln	Val	
					185					190					195		
	CTC	CAG	GAG	CAC	TCA	GGC	AGG	GAG	TCG	GAC	CTC	TTC	TTG	CTG	GAC	AGC	739
	Leu	Gln	Glu	His	Ser	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	Asp	Ser	
					200				205					210			
45	CGC	ACC	ATC	TGG	GCT	TCT	GAG	GAG	GGC	TGG	TTG	GTG	TTT	GAT	ATC	ACA	787
	Arg	Thr	Ile	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	Ile	Thr	
			215					220					225				
50	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAC	CCT	CGG	CAC	AAC	CTG	GGC	TTA	835
	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	Gly	Leu	
						230		235				240					
55	CAG	CTC	TCT	GTG	GAG	ACC	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	AAG	TTG	883
	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	Lys	Leu	
	245					250					255					260	

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	GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG	931
	Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met	
	265 270 275	
5	GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC	979
	Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser	
	280 285 290	
10	ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC	1027
	Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn	
	295 300 305	
15	CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC	1075
	Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp	
	310 315 320	
20	CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC	1123
	Gln Arg Gln Ala Cys Lys His Glu Leu Tyr Val Ser Phe Arg Asp	
	325 330 335 340	
	CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC	1171
	Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr	
	345 350 355	
25	TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC	1219
	Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala	
	360 365 370	
30	ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC	1267
	Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp	
	375 380 385	
35	ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT	1315
	Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser	
	390 395 400	
40	GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC GAC CTG AAG AAG TAC AGA	1363
	Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Asp Leu Lys Lys Tyr Arg	
	405 410 415 420	
	AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCTTG	1413
	Asn Met Val Val Arg Ala Cys Gly Cys His	
	425 430	
45	ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG	1473
	CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG	1533
50	AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCTTCT	1593
	GGCAGCTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT	1653
	GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCTGGC GCTCTGAGTC TTGAGGAGT	1713

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AATCGCAAGC CTCGTCAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GCGCTGGCG 1773
 TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAACCCAT 1833
 5 GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTG 1873

(2) INFORMATION FOR SEQ ID NO:19:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 430 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 20 1 5 10 15
 Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
 25 20 25 30
 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
 35 40 45
 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
 50 55 60
 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
 65 70 75 80
 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
 35 85 90 95
 Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
 100 105 110
 Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
 40 115 120 125
 Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
 130 135 140
 Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
 145 150 155 160
 Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
 50 165 170 175
 Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
 180 185 190
 55 Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
 195 200 205

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Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
 210 215 220
 5 Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
 225 230 235 240
 Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
 245 250 255
 10 Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
 260 265 270
 Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
 15 275 280 285
 Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
 290 295 300
 20 Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
 305 310 315 320
 Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
 325 330 335
 25 Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
 340 345 350
 Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
 30 355 360 365
 Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
 370 375 380
 35 Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
 385 390 395 400
 Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Asp Leu
 405 410 415
 40 Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:20:

45

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1723 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

55

- (A) ORGANISM: Homo sapiens
 (E) TISSUE TYPE: HIPPOCAMPUS

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(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 490..1696
 5 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "hOP2-PP"
 /note= "hOP2 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGCGCCGGCA	GAGCAGGAGT	GGCTGGAGGA	GCTGTGGTTG	GAGCAGGAGG	TGGCACGGCA	60
GGGCTGGAGG	GCTCCCTATG	AGTGGCGGAG	ACGGCCCAGG	AGGCGCTGGA	GCAACAGCTC	120
15 CCACACCGCA	CCAAGCGGTG	GCTGCAGGAG	CTCGCCCATC	GCCCCTGCGC	TGCTCGGACC	180
GCGGCCACAG	CCGGA CTGGC	GGGTACGGCG	GCGACAGAGG	CATTGGCCGA	GAGTCCCAGT	240
20 CCGCAGAGTA	GCCCCGGCCT	CGAGGCGGTG	GCGTCCCGGT	CCTCTCCGTC	CAGGAGCCAG	300
GACAGGTGTC	GCGCGGCGGG	GCTCCAGGGA	CCGCGCTTGA	GGCCGGCTGC	CCGCCCGTCC	360
CGCCCCGCC	CGCGCCCCG	CGCCCCCGGA	GCCCCAGCCTC	CTTGCCGTCG	GGGCGTCCCC	420
25 AGGCCCTGGG	TCGGCCGCGG	AGCCGATGCG	CGCCCCGCTGA	GCGCCCCAGC	TGAGCGCCCC	480
CGGCCTGCC	ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG					528
30	Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu					
	1 5 10					
GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC						576
Ala Leu Cys Ala Leu Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro						
15 20 25						
35 GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG						624
Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln						
30 35 40 45						
40 CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC						672
Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg						
50 55 60						
GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG						720
Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met						
65 70 75						
CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG						768
Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala						
80 85 90						
CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT						816
Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val						
95 100 105						

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	AAC	ATG	GTG	GAG	CGA	GAC	CGT	GCC	CTG	GGC	CAC	CAG	GAG	CCC	CAT	TGG	864
	Asn	Met	Val	Glu	Arg	Asp	Arg	Ala	Leu	Gly	His	Gln	Glu	Pro	His	Trp	
	110					115					120					125	
5	AAG	GAG	TTC	CGC	TTT	GAC	CTG	ACC	CAG	ATC	CCG	GCT	GGG	GAG	GCG	GTC	912
	Lys	Glu	Phe	Arg	Phe	Asp	Leu	Thr	Gln	Ile	Pro	Ala	Gly	Glu	Ala	Val	
					130					135					140		
10	ACA	GCT	GCG	GAG	TTC	CGG	ATT	TAC	AAG	GTG	CCC	AGC	ATC	CAC	CTG	CTC	960
	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Val	Pro	Ser	Ile	His	Leu	Leu	
					145					150					155		
15	AAC	AGG	ACC	CTC	CAC	GTC	AGC	ATG	TTC	CAG	GTG	GTC	CAG	GAG	CAG	TCC	1008
	Asn	Arg	Thr	Leu	His	Val	Ser	Met	Phe	Gln	Val	Val	Gln	Glu	Gln	Ser	
			160					165						170			
	AAC	AGG	GAG	TCT	GAC	TTG	TTC	TTT	TTG	GAT	CTT	CAG	ACG	CTC	CGA	GCT	1056
	Asn	Arg	Glu	Ser	Asp	Leu	Phe	Phe	Leu	Asp	Leu	Gln	Thr	Leu	Arg	Ala	
			175				180						185				
20	GGA	GAC	GAG	GGC	TGG	CTG	GTG	CTG	GAT	GTC	ACA	GCA	GCC	AGT	GAC	TGC	1104
	Gly	Asp	Glu	Gly	Trp	Leu	Val	Leu	Asp	Val	Thr	Ala	Ala	Ser	Asp	Cys	
						195					200					205	
25	TGG	TTG	CTG	AAG	CGT	CAC	AAG	GAC	CTG	GGA	CTC	CGC	CTC	TAT	GTG	GAG	1152
	Trp	Leu	Leu	Lys	Arg	His	Lys	Asp	Leu	Gly	Leu	Arg	Leu	Tyr	Val	Glu	
					210					215					220		
30	ACT	GAG	GAC	GGG	CAC	AGC	GTG	GAT	CCT	GGC	CTG	GCC	GGC	CTG	CTG	GGT	1200
	Thr	Glu	Asp	Gly	His	Ser	Val	Asp	Pro	Gly	Leu	Ala	Gly	Leu	Leu	Gly	
					225				230						235		
35	CAA	CGG	GCC	CCA	CGC	TCC	CAA	CAG	CCT	TTC	GTG	GTC	ACT	TTC	TTC	AGG	1248
	Gln	Arg	Ala	Pro	Arg	Ser	Gln	Gln	Pro	Phe	Val	Val	Thr	Phe	Phe	Arg	
			240					245						250			
	GCC	AGT	CCG	AGT	CCC	ATC	CGC	ACC	CCT	CGG	GCA	GTG	AGG	CCA	CTG	AGG	1296
	Ala	Ser	Pro	Ser	Pro	Ile	Arg	Thr	Pro	Arg	Ala	Val	Arg	Pro	Leu	Arg	
		255					260						265				
40	AGG	AGG	CAG	CCG	AAG	AAA	AGC	AAC	GAG	CTG	CCG	CAG	GCC	AAC	CGA	CTC	1344
	Arg	Arg	Gln	Pro	Lys	Lys	Ser	Asn	Glu	Leu	Pro	Gln	Ala	Asn	Arg	Leu	
						275					280					285	
45	CCA	GGG	ATC	TTT	GAT	GAC	GTC	CAC	GGC	TCC	CAC	GGC	CGG	CAG	GTC	TGC	1392
	Pro	Gly	Ile	Phe	Asp	Val	His	Gly	Ser	His	His	Gly	Arg	Gln	Val	Cys	
					290					295					300		
50	CGT	CGG	CAC	GAG	CTC	TAC	GTC	AGC	TTC	CAG	GAC	CTC	GGC	TGG	CTG	GAC	1440
	Arg	Arg	His	Glu	Leu	Tyr	Val	Ser	Phe	Gln	Asp	Leu	Gly	Trp	Leu	Asp	
					305					310					315		
55	TGG	GTC	ATC	GCT	CCC	CAA	GGC	TAC	TCG	GCC	TAT	TAC	TGT	GAG	GGG	GAG	1488
	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr	Glu	Gly	Glu		
			320					325						330			

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	TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC	1536
	Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile	
	335 340 345	
5	CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG	1584
	Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala	
	350 355 360 365	
10	TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC	1632
	Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp	
	370 375	
15	AGC AGC AAC AAC GTC ATC CTG CGC AAA GCC CGC AAC ATG GTG GTC AAG	1680
	Ser Ser Asn Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys	
	385 390 395	
20	GCC TGC GGC TGC CAC T GAGTCAGCCCC GCCCAGCCCT ACTGCAG	1723
	Ala Cys Gly Cys His	
	400	

(2) INFORMATION FOR SEQ ID NO:21:

25	(i) SEQUENCE CHARACTERISTICS:
	(A) LENGTH: 402 amino acids
	(B) TYPE: amino acid
	(D) TOPOLOGY: linear
30	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
35	Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
	1 5 10 15
	Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro
	20 25 30
40	Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile
	35 40 45
	Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
	50 55 60
45	Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
	65 70 75 80
50	Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
	85 90 95
	Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
	100 105 110
55	Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
	115 120 125

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Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
 130 135 140
 5 Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
 145 150 155 160
 Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
 165 170 175
 10 Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
 180 185 190
 Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
 195 200 205
 15 Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
 210 215 220
 20 Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
 225 230 235 240
 Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
 245 250 255
 25 Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Gln
 260 265 270
 Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
 275 280 285
 30 Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
 290 295 300
 35 Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
 305 310 315 320
 Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
 325 330 335
 40 Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
 340 345 350
 Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
 355 360 365
 45 Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
 370 375 380
 50 Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly
 385 390 395 400
 Cys His

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(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1926 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
 (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 93..1289
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "mOP2-PP"
 /note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

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GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC CCGACCAGCT      60
25 ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG  ATG  GCT  ATG  CGT  CCC  GGG  CCA      113
                                     Met Ala Met Arg Pro Gly Pro
                                     1          5

CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC GGC CAC GGT      161
30 Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly His Gly
    10          15          20

CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA GCG CGC GAG      209
35 Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu
    25          30          35

CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG CTA CCG GGA      257
40 Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly
    40          45          50          55

CGG CCC CGA CCC CGT GCA CAA CCC GCC GCT GCC CGG CAG CCA GCG TCC      305
Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln Pro Ala Ser
    60          65          70

GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC GAC      353
45 Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp
    75          80          85

GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC ATG      401
50 Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val Met
    90          95          100

AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG GAG      449
55 Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln Glu
    105          110          115

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	CCA	CAC	TGG	AAG	GAA	TTC	CAC	TTT	GAC	CTA	ACC	CAG	ATC	CCT	GCT	GGG	497
	Pro	His	Trp	Lys	Glu	Phe	His	Phe	Asp	Leu	Thr	Gln	Ile	Pro	Ala	Gly	
	120					125					130					135	
5	GAG	GCT	GTC	ACA	GCT	GCT	GAG	TTC	CGG	ATC	TAC	AAA	GAA	CCC	AGC	ACC	545
	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Glu	Pro	Ser	Thr	
				140					145					150			
10	CAC	CCG	CTC	AAC	ACA	ACC	CTC	CAC	ATC	AGC	ATG	TTC	GAA	GTG	GTC	CAA	593
	His	Pro	Leu	Asn	Thr	Thr	Leu	His	Ile	Ser	Met	Phe	Glu	Val	Val	Gln	
				155					160					165			
15	GAG	CAC	TCC	AAC	AGG	GAG	TCT	GAC	TTG	TTC	TTT	TTG	GAT	CTT	CAG	ACG	641
	Glu	His		Asn	Arg	Glu	Ser	Asp	Leu	Phe	Phe	Leu	Asp	Leu	Gln	Thr	
			170					175					180				
20	CTC	CGA	TCT	GGG	GAC	GAG	GGC	TGG	CTG	GTG	CTG	GAC	ATC	ACA	GCA	GCC	689
	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu	Val	Leu	Asp	Ile	Thr	Ala	Ala	
		185					190					195					
25	AGT	GAC	CGA	TGG	CTG	CTG	AAC	CAT	CAC	AAG	GAC	CTG	GGA	CTC	CGC	CTC	737
	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His	Lys	Asp	Leu	Gly	Leu	Arg	Leu	
	200				205					210						215	
30	TAT	GTG	GAA	ACC	GCG	GAT	GGG	CAC	AGC	ATG	GAT	CCT	GGC	CTG	GCT	GGT	785
	Tyr	Val	Glu	Thr	Ala	Asp	Gly	His	Ser	Met	Asp	Pro	Gly	Leu	Ala	Gly	
				220						225				230			
35	CTG	CTT	GGA	CGA	CAA	GCA	CCA	CGC	TCC	AGA	CAG	CCT	TTC	ATG	GTA	ACC	833
	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser	Arg	Gln	Pro	Phe	Met	Val	Thr	
			235					240						245			
40	TTC	TTC	AGG	GCC	AGC	CAG	AGT	CCT	GTG	CGG	GCC	CCT	CGG	GCA	GCG	AGA	881
	Phe	Phe	Arg	Ala	Ser	Gln	Ser	Pro	Val	Arg	Ala	Pro	Arg	Ala	Ala	Arg	
			250					255					260				
45	CCA	CTG	AAG	AGG	AGG	CAG	CCA	AAG	AAA	ACG	AAC	GAG	CTT	CCG	CAC	CCC	929
	Pro	Leu	Lys	Arg	Arg	Gln	Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His	Pro	
		265					270					275					
50	AAC	AAA	CTC	CCA	GGG	ATC	TTT	GAT	GAT	GGC	CAC	GGT	TCC	CGC	GGC	AGA	977
	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp	Asp	Gly	His	Gly	Ser	Arg	Gly	Arg	
	280					285					290					295	
55	GAG	GTT	TGC	CGC	AGG	CAT	GAG	CTC	TAC	GTC	AGC	TTC	CGT	GAC	CTT	GGC	1025
	Glu	Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	Asp	Leu	Gly	
				300					305					310			
60	TGG	CTG	GAC	TGG	GTC	ATC	GCC	CCC	CAG	GGC	TAC	TCT	GCC	TAT	TAC	TGT	1073
	Trp	Leu	Asp	Trp	Val	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	
				315				320						325			
65	GAG	GGG	GAG	TGT	GCT	TTC	CCA	CTG	GAC	TCC	TGT	ATG	AAC	GCC	ACC	AAC	1121
	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asp	Ser	Cys	Met	Asn	Ala	Thr	Asn	
			330					335					340				

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	CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC	1169
	His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val	
	345 350 355	
5	CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG	1217
	Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu	
	360 365 370 375	
10	TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG	1265
	Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met	
	380 385 390	
	GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCG CCCAGCATCC TGCTTCTACT	1319
15	Val Val Lys Ala Cys Gly Cys His	
	395	
	ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT	1379
20	CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCCTGCTA AAATTCTGGT	1439
	CTTTCCCACT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC CTCTCCATCC	1499
	TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT	1559
25	CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC CTCAGCCAC	1619
	AATGGCAAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTC TAACTAGAT GATCTGGGCT	1679
30	CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTTAGGT ATAACAGACA CATACACTTA	1739
	GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAATCAGAG	1799
	CCAGGTATAG CGGTGCATGT CATTAAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAATCT	1859
35	CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAAAC	1919
	GGAATTC	1926

40

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 399 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met	Ala	Met	Arg	Pro	Gly	Pro	Leu	Trp	Leu	Leu	Gly	Leu	Ala	Leu	Cys
1						5			10					15	

55	Ala	Leu	Gly	Gly	Gly	His	Gly	Pro	Arg	Pro	Pro	His	Thr	Cys	Pro	Gln
				20					25					30		

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[illegible]

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	Gly	Tyr	Ser	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asp
					325					330					335	
5	Ser	Cys	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Leu	Gln	Ser	Leu	Val	His
				340					345					350		
	Leu	Met	Lys	Pro	Asp	Val	Val	Pro	Lys	Ala	Cys	Cys	Ala	Pro	Thr	Lys
			355					360					365			
10	Leu	Ser	Ala	Thr	Ser	Val	Leu	Tyr	Tyr	Asp	Ser	Ser	Asn	Asn	Val	Ile
		370					375					380				
	Leu	Arg	Lys	His	Arg	Asn	Met	Val	Val	Lys	Ala	Cys	Gly	Cys	His	
	385					390					395					

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1368 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) **FEATURE:**

- (A) NAME/KEY: CDS
(B) LOCATION: 1..1368

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

35	ATG Met	TCG Ser	GGA Gly	CTG Leu	CGA Arg	AAC Asn	ACC Thr	TCG Ser	GAG Glu	GCC Ala	GTT Val	GCA Ala	GTG Val	CTC Leu	GCC Ala	TCC Ser	48
	1				5					10					15		
40	CTG Leu	GGA Gly	CTC Leu	GGA Gly	ATG Met	GTT Val	CTG Leu	CTC Leu	ATG Met	TTC Phe	GTG Val	GCG Ala	ACC Thr	ACG Thr	CCG Pro	CCG Pro	96
				20					25					30			
45	GCC Ala	GTT Val	GAG Glu	GCC Ala	ACC Thr	CAG Gln	TCG Ser	GGG Gly	ATT Ile	TAC Tyr	ATA Ile	GAC Asp	AAC Asn	GGC Gly	AAG Lys	GAC Asp	144
				35				40					45				
	CAG Gln	ACG Thr	ATC Ile	ATG Met	CAC His	AGA Arg	GTG Val	CTG Leu	AGC Ser	GAG Glu	GAC Asp	GAC Asp	AAG Lys	CTG Leu	GAC Asp	GTC Val	192
				50			55					60					
50	TCG Ser	TAC Tyr	GAG Glu	ATC Ile	CTC Leu	GAG Glu	TTC Phe	CTG Leu	GGC Gly	ATC Ile	GCC Ala	GAA Glu	CGG Arg	CCG Pro	ACG Thr	CAC His	240
	65					70					75				80		
55	CTG Leu	AGC Ser	AGC Ser	CAC His	CAG Gln	TTG Leu	TCG Ser	CTG Leu	AGG Arg	AAG Lys	TCG Ser	GCT Ala	CCC Pro	AAG Lys	TTC Phe	CTG Leu	288

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	CTG	GAC	GTC	TAC	CAC	CGC	ATC	ACG	GCG	GAG	GAG	GGT	CTC	AGC	GAT	CAG	336
	Leu	Asp	Val	Tyr	His	Arg	Ile	Thr	Ala	Glu	Glu	Gly	Leu	Ser	Asp	Gln	
				100					105					110			
5	GAT	GAG	GAC	GAC	GAC	TAC	GAA	CGC	GGC	CAT	CGG	TCC	AGG	AGG	AGC	GCC	384
	Asp	Glu	Asp	Asp	Asp	Tyr	Glu	Arg	Gly	His	Arg	Ser	Arg	Arg	Ser	Ala	
				115				120					125				
10	GAC	CTC	GAG	GAG	GAT	GAG	GGC	GAG	CAG	CAG	AAG	AAC	TTC	ATC	ACC	GAC	432
	Asp	Leu	Glu	Glu	Asp	Glu	Gly	Glu	Gln	Gln	Lys	Asn	Phe	Ile	Thr	Asp	
				130			135					140					
15	CTG	GAC	AAG	CGG	GCC	ATC	GAC	GAG	AGC	GAC	ATC	ATC	ATG	ACC	TTC	CTG	480
	Leu	Asp	Lys	Arg	Ala	Ile	Asp	Glu	Ser	Asp	Ile	Ile	Met	Thr	Phe	Leu	
					150						155					160	
20	AAC	AAG	CGC	CAC	CAC	AAT	GTG	GAC	GAA	CTG	CGT	CAC	GAG	CAC	GGC	CGT	528
	Asn	Lys	Arg	His	His	Asn	Val	Asp	Glu	Leu	Arg	His	Glu	His	Gly	Arg	
					165					170					175		
25	CGC	CTG	TGG	TTC	GAC	GTC	TCC	AAC	GTG	CCC	AAC	GAC	AAC	TAC	CTG	GTG	576
	Arg	Leu	Trp	Phe	Asp	Val	Ser	Asn	Val	Pro	Asn	Asp	Asn	Tyr	Leu	Val	
				180					185					190			
30	ATG	GCC	GAG	CTG	CGC	ATC	TAT	CAG	AAC	GCC	AAC	GAG	GGC	AAG	TGG	CTG	624
	Met	Ala	Glu	Leu	Arg	Ile	Tyr	Gln	Asn	Ala	Asn	Glu	Gly	Lys	Trp	Leu	
				195				200					205				
35	ACC	GCC	AAC	AGG	GAG	TTC	ACC	ATC	ACG	GTA	TAC	GCC	ATT	GGC	ACC	GGC	672
	Thr	Ala	Asn	Arg	Glu	Phe	Thr	Ile	Thr	Val	Tyr	Ala	Ile	Gly	Thr	Gly	
				210			215					220					
40	ACG	CTG	GGC	CAG	CAC	ACC	ATG	GAG	CCG	CTG	TCC	TCG	GTG	AAC	ACC	ACC	720
	Thr	Leu	Gly	Gln	His	Thr	Met	Glu	Pro	Leu	Ser	Ser	Val	Asn	Thr	Thr	
						230					235					240	
45	GGG	GAC	TAC	GTG	GGC	TGG	TTG	GAG	CTC	AAC	GTG	ACC	GAG	GGC	CTG	CAC	768
	Gly	Asp	Tyr	Val	Gly	Trp	Leu	Glu	Leu	Val	Thr	Glu	Gly	Gly	Thr	His	
				245						250					255		
50	GAG	TGG	CTG	GTC	AAG	TCG	AAG	GAC	AAT	CAT	GGC	ATC	TAC	ATT	GGA	GCA	816
	Glu	Trp	Leu	Val	Lys	Ser	Lys	Asp	Asn	His	Gly	Ile	Tyr	Ile	Gly	Ala	
				260				265					270				
55	CAC	GCT	GTC	AAC	CGA	CCC	GAC	CGC	GAG	GTG	AAG	CTG	GAC	GAC	ATT	GGA	864
	His	Ala	Val	Asn	Arg	Pro	Asp	Arg	Glu	Val	Lys	Leu	Asp	Asp	Ile	Gly	
				275			280						285				
60	CTG	ATC	CAC	CGC	AAG	GTG	GAC	GAC	GAG	TTC	CAG	CCC	TTC	ATG	ATC	GGC	912
	Leu	Ile	His	Arg	Lys	Val	Asp	Asp	Glu	Phe	Gln	Pro	Phe	Met	Ile	Gly	
						290	295					300					
65	TTC	TTC	CGC	GGA	CCG	GAG	CTG	ATC	AAG	GCG	ACG	GCC	CAC	AGC	AGC	CAC	960
	Phe	Phe	Arg	Gly	Pro	Glu	Leu	Ile	Lys	Ala	Thr	Ala	His	Ser	Ser	His	
						310					315					320	

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	CAC AGG AGC AAG CGA AGC GCC AGC CAT CCA CGC AAG CGC AAG AAG TCG	1008
	His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser	
	325 330 335	
5	GTG TCG CCC AAC AAC GTG CCG CTG CTG GAA CCG ATG GAG AGC ACG CGC	1056
	Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg	
	340 345 350	
10	AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG	1104
	Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp	
	355 360 365	
15	CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC	1152
	His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser	
	370 375 380	
20	GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT	1200
	Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His	
	385 390 395 400	
25	GCG ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC	1248
	Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro	
	405 410 415	
30	AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC	1296
	Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr	
	420 425 430	
35	CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT	1344
	His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile	
	435 440 445	
40	GTG AAA TCC TGC GGG TGC CAT TGA	1368
	Val Lys Ser Cys Gly Cys His	
	450 455	

(2) INFORMATION FOR SEQ ID NO:25:

40

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 455 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

50	Met	Ser	Gly	Leu	Arg	Asn	Thr	Ser	Glu	Ala	Val	Ala	Val	Leu	Ala	Ser
	1				5					10				15		
	Leu	Gly	Leu	Gly	Met	Val	Leu	Leu	Met	Phe	Val	Ala	Thr	Thr	Pro	Pro
				20					25					30		

55

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Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp
35 40 45

5 Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val
50 55 60

Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His
65 70 75 80

10 Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu
85 90 95

Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln
100 105 110

15 Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala
115 120 125

20 Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
130 135 140

Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
145 150 155 160

25 Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
165 170 175

Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
180 185 190

30 Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
195 200 205

35 Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
210 215 220

Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr
225 230 235 240

40 Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
245 250 255

Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
260 265 270

45 His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
275 280 285

50 Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
290 295 300

Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
305 310 315 320

55 His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
325

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Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
 340 345 350

5 Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
 355 360 365

His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
 370 375 380

10 Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
 385 390 395 400

Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
 405 410 415

15 Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
 420 425 430

20 His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
 435 440 445

Val Lys Ser Cys Gly Cys His
 450 455

25

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
 30 (A) LENGTH: 104 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(ix) FEATURE:
 40 (A) NAME/KEY: Protein
 (B) LOCATION: 1..104
 (D) OTHER INFORMATION: /note= "BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

45 Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
 1 5 10 15

50 Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly
 20 25 30

Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
 35 40 45

55 Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile
 55 60

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Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
65 70 75 80

5 Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
85 90 95

Thr Val Glu Ser Cys Ala Cys Arg
100

10 (2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
15 (A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20 (vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:
25 (A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /note= "BMP5"

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln
1 5 10 15

35 Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly
20 25 30

Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35 40 45

40 Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
50 55 60

45 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65 70 75 80

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
85 90 95

50 Arg Ser Cys Gly Cys His
100

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(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS
- (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..102
 (D) OTHER INFORMATION: /note= "BMP6"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
- | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Arg | Lys | His | Glu | Leu | Tyr | Val | Ser | Phe | Gln | Asp | Leu | Gly | Trp | Gln |
| 1 | | | | 5 | | | | | 10 | | | | 15 | | |
| Asp | Trp | Ile | Ile | Ala | Pro | Lys | Gly | Tyr | Ala | Ala | Asn | Tyr | Cys | Asp | Gly |
| | | 20 | | | | | 25 | | | | | 30 | | | |
| Glu | Cys | Ser | Phe | Pro | Leu | Asn | Ala | His | Met | Asn | Ala | Thr | Asn | His | Ala |
| | | 35 | | | | 40 | | | | | 45 | | | | |
| Ile | Val | Gln | Thr | Leu | Val | His | Leu | Met | Asn | Pro | Glu | Tyr | Val | Pro | Lys |
| | | 50 | | | | 55 | | | | | 60 | | | | |
| Pro | Cys | Cys | Ala | Pro | Thr | Lys | Leu | Asn | Ala | Ile | Ser | Val | Leu | Tyr | Phe |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Asp | Asp | Asn | Ser | Asn | Val | Ile | Leu | Lys | Lys | Tyr | Arg | Trp | Met | Val | Val |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Arg | Ala | Cys | Gly | Cys | His | | | | | | | | | | |
| | | | | 100 | | | | | | | | | | | |

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

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(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= OPX

5 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
AS DEFINED IN THE SPECIFICATION (SECTION II.B.2.)"

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
1 5 10 15

15 Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
20 25 30

Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
35 40 45

20 Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
50 55 60

25 Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65 70 75 80

Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
85 90 95

30 Xaa Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:30:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..97

(D) OTHER INFORMATION: /label= GENERIC-SEQ5

45 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
50 AS DEFINED IN THE SPECIFICATION."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

55 Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa Xaa Xaa

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Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Met Xaa Val
85 90 95

5 Xaa Xaa Cys Xaa Cys Xaa
100

(2) INFORMATION FOR SEQ ID NO:32:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1247 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
20 (F) TISSUE TYPE: BRAIN

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 84..1199
25 (D) OTHER INFORMATION: /product= "GDF-1"
/note= "GDF-1 CDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

30 GGGGACACCG GCCCGCCCT CAGCCCACTG GTCCCGGGCC GCCCGGGACC CTGCGCACTC 60
TCTGTGCATC GCCTGGGAGG AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC 110
Met Pro Pro Pro Gln Gln Gly Pro Cys
35 1 5
GGC CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC 158
Gly His His Leu Leu Leu Leu Leu Ala Leu Leu Pro Ser Leu Pro 25
10 15 20
40 CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC CAG 206
Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu Gln 30 35 40
45 GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC CGG CCG 254
Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu Arg Pro 45 50 55
GTI CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC CCC CAG GAG 302
50 Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp Pro Gln Glu 60 65 70
ACC AGG TCT GGC TCG CGG CGG ACG TCC CCA GGG GTC ACC CTG CAA CCG 350
Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val Thr Leu Gln Pro 75 80 85

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	TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC ATC GTG CGC CAC ATC	398
	Cys His Val Glu Glu Leu Gly Val Ala Gly Asn Ile Val Arg His Ile	
	90 95 100 105	
5	CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG GAG CCT GTC TCG GCC GCG	446
	Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser Glu Pro Val Ser Ala Ala	
	110 115 120	
10	GGG CAT TGC CCT GAG TGG ACA GTC GTC TTC GAC CTG TCG GCT GTG GAA	494
	Gly His Cys Pro Glu Trp Thr Val Phe Asp Leu Ser Ala Val Glu	
	125 130 135	
15	CCC GCT GAG CGC CCG AGC CGG GCC CGC CTG GAG CTG CGT TTC GCG GCG	542
	Pro Ala Glu Arg Pro Ser Arg Ala Arg Leu Glu Leu Arg Phe Ala Ala	
	140 145 150	
20	GCG GCG GCG GCA GCC CCG GAG GGC GGC TGG GAG CTG AGC GTG GCG CAA	590
	Ala Ala Ala Ala Ala Pro Glu Gly Trp Glu Ser Val Ala Gln	
	155 160 165	
25	GCG GGC CAG GGC GCG GGC GCG GAC CCC GGG CCG GTG CTG CTC GCG CAG	638
	Ala Gly Gln Gly Ala Gly Ala Asp Pro Gly Pro Val Leu Leu Arg Gln	
	170 175 180 185	
30	TTG GTG CCC GCC CTG GGG CCG CCA GTG CGC GCG GAG CTG CTG GGC GCC	686
	Leu Val Pro Ala Leu Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala	
	190 195 200	
35	GCT TGG GCT CGC AAC GCC TCA TGG CCG CGC AGC CTC CGC CTG GCG CTG	734
	Ala Trp Ala Ala Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu	
	205 210 215	
40	GCG CTA CGC CCC CGG GCC CCT GCC GCC TGC GCG CGC CTG GCC GAG GCC	782
	Ala Leu Arg Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala	
	220 225 230	
45	TCG CTG CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC	830
	Ser Leu Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala	
	235 240 245	
50	CGG CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC	878
	Arg Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly	
	250 255 260 265	
55	GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC GAG GTG GGC TGG	926
	Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp	
	270 275 280	
60	CAC CGC TGG GTC ATC CCG CCG CGC GGC TTC CTG GCC AAC TAC TGC CAG	974
	His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln	
	285 290 295	
65	GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG GGC CCG CCG	1022
	Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro	
	300 305 310	

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GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC GCG GCC GCC CCG 1070
 Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Pro
 315 320 325

5

GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG CGC CTG TCG CCC ATC 1118
 Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile
 330 335 340 345

10

TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC GTG GTG CTG CGG CAG TAT 1166
 Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr
 350 355 360

15

GAG GAC ATG GTG GTG GAC GAG TGC GGC TGC CGC TAACCCGGGG CGGGCAGGGA 1219
 Glu Asp Met Val Val Asp Glu Cys Gly Cys Arg
 365 370

CCCGGGCCCA ACAATAAATG CCGCGTGG 1247

20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

25

(A) LENGTH: 372 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Pro Pro Pro Gln Gln Gly Pro Cys Gly His His Leu Leu Leu Leu
 1 5 10 15

35

Leu Ala Leu Leu Leu Pro Ser Leu Pro Leu Thr Arg Ala Pro Val Pro
 20 25 30

Pro Gly Pro Ala Ala Ala Leu Leu Gln Ala Leu Gly Leu Arg Asp Glu
 35 40 45

40

Pro Gln Gly Ala Pro Arg Leu Arg Pro Val Pro Pro Val Met Trp Arg
 50 55 60

45

Leu Phe Arg Arg Arg Asp Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg
 65 70 75 80

Thr Ser Pro Gly Val Thr Leu Gln Pro Cys His Val Glu Glu Leu Gly
 85 90 95

50

Val Ala Gly Asn Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr
 100 105 110

Arg Ala Ser Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr
 115 120 125

55

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Val Val Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg
 130 135 140
 Ala Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Pro Glu
 5 145 150 155 160
 Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly Ala
 165 170 175
 10 Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu Gly Pro
 180 185 190
 Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg Asn Ala Ser
 195 200 205
 15 Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg Pro Arg Ala Pro
 210 215 220
 Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu Leu Leu Val Thr Leu
 20 225 230 235 240
 Asp Pro Arg Leu Cys His Pro Leu Ala Arg Pro Arg Arg Asp Ala Glu
 245 250 255
 25 Pro Val Leu Gly Gly Gly Pro Gly Gly Ala Cys Arg Ala Arg Arg Leu
 260 265 270
 Tyr Val Ser Phe Arg Glu Val Gly Trp His Arg Trp Val Ile Ala Pro
 275 280 285
 30 Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly Gln Cys Ala Leu Pro Val
 290 295 300
 Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala Leu Asn His Ala Val Leu
 35 305 310 315 320
 Arg Ala Leu Met His Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys
 325 330 335
 40 Cys Val Pro Ala Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn
 340 345 350
 Ser Asp Asn Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu
 355 360 365
 45 Cys Gly Cys Arg
 370

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What is claimed is:

- 1 1. A method for maintaining normal liver function
2 following hepatic tissue injury in a mammal or in
3 anticipation of such injury, the method comprising
4 the step of providing to said liver a
5 therapeutically effective concentration of a
6 morphogen.
- 1 2. A method for enhancing the level of a depressed
2 liver function in a mammal, said liver function
3 being depressed due to a tissue injury or disease,
4 the method comprising the step of providing to said
5 liver a therapeutically effective concentration of
6 a morphogen.
- 1 3. The method of claim 1 or 2 wherein said step of
2 providing a therapeutically effective morphogen
3 concentration comprises the step of administering a
4 therapeutically effective concentration of a
5 morphogen to said mammal.
- 1 4. The method of claim 1 or 2 wherein said step of
2 providing a therapeutically effective morphogen
3 concentration comprises the step of administering
4 to said mammal an agent that stimulates in vivo a
5 therapeutically effective concentration of an
6 endogenous morphogen.
- 1 5. The method of claim 1 or 2 wherein said liver
2 function is reduced due to a hepatocellular injury.
- 1 6. The method of claim 5 wherein the etiology of said
2 hepatocellular injury is metabolic, infectious,
3 toxic, autoimmune, ischemic or nutritional.

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- 1 7. The method of claim 6 wherein said hepatocellular
2 injury comprises hyperbilirubinemia, viral
3 hepatitis, alcoholic liver disease, portal
4 hypertension, neonatal hepatitis or hepatic
5 encephalopathy.
- 1 8. The method of claim 1 or 2 wherein said liver
2 function is reduced due to liver cirrhosis.
- 1 9. The method of claim 1 or 2 wherein said liver
2 function is reduced due to a neoplasm.
- 1 10. The method of claim 9 wherein said neoplasm
2 comprises hepatocytes.
- 1 11. The method of claim 10 wherein said neoplasm
2 comprises a hepatic adenoma, nodular hyperplasia,
3 hepatocellular carcinoma or a hemangiosarcoma.
- 1 12. The method of claim 9 wherein said neoplasm
2 comprises cells of a metastatic cancer.
- 1 13. The method of claim 12 wherein said metastatic
2 cancer originated in tissue of the gastrointestinal
3 tract, breast, lung or skin.
- 1 14. The method of claim 1 or 2 wherein said liver is at
2 risk of hepatic failure.
- 1 15. The method of claim 5 wherein said tissue injury
2 results from toxic concentrations of ammonia,
3 phenol, ethanol, infectious agent byproduct, carbon
4 tetrachloride or a metal.

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- 1 16. The method of claim 5 wherein said tissue injury
2 results from a toxic concentration of a
3 pharmaceutical agent or its metabolite.
- 1 17. The method of claim 1 or 2 wherein said tissue
2 injury is induced in a clinical procedure.
- 1 18. The method of claim 17 wherein said tissue injury
2 is induced in a surgical procedure.
- 1 19. A method for inducing regeneration of lost or
2 damaged hepatic tissue in a mammal, the method
3 comprising the step of:
4 providing to the locus of said damaged or lost
5 tissue, a therapeutically effective concentration
6 of a morphogen.
- 1 20. The method of claim 19 wherein said morphogen is
2 provided to said locus in association with a
3 biocompatible, acellular matrix.
- 1 21. The method of claim 20 wherein said matrix has
2 components specific for said tissue.
- 1 22. The method of claim 20 wherein said matrix is
2 biodegradable.
- 1 23. The method of claim 20 wherein said matrix is
2 derived from organ-specific tissue.
- 1 24. The method of claim 20 wherein said matrix
2 comprises collagen and cell attachment factors
3 specific for said tissue.

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- 1 25. The method of claim 20 wherein said matrix
2 comprises a synthetic polymeric material.
- 1 26. The method of claim 20 wherein said matrix defines
2 pores of a dimension sufficient to permit the
3 influx, differentiation and proliferation of
4 migratory progenitor cells from the body of said
5 mammal.
- 1 27. The method of claim 25 wherein said polymeric
2 material comprises polylactic acid, polybutyric
3 acid, polyglycolic acid, polyanhydride, or
4 copolymers thereof.
- 1 28. A method for inducing hepatic tissue formation in a
2 mammal, said method comprising the steps of:
3 a) stimulating progenitor cells by exposure
4 to a therapeutically effective morphogen
5 concentration,
6 b) implanting said stimulated cells at a
7 liver-specific locus in vivo, such that said
8 stimulated cells are capable of proliferation and
9 differentiation at said locus.
- 1 29. The method of claim 28 wherein said progenitor
2 cells are of mesenchymal origin.
- 1 30. The method of claim 28 wherein said stimulated
2 cells are implanted at said locus, in association
3 with a biocompatible, acellular matrix.

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- 1 31. A method for enhancing integration of a liver
2 tissue transplant, the method comprising the step
3 of providing a therapeutically effective
4 concentration of a morphogen to the liver tissue
5 transplant locus.
- 1 32. The method of claim 31 wherein said morphogen is
2 provided to said locus prior to transplantation.
- 1 33. The method of claim 31 wherein said morphogen is
2 provided to said locus concurrent with
3 transplantation.
- 1 34. A method for enhancing integration of a liver
2 tissue transplant, the method comprising the step
3 of providing a therapeutically effective
4 concentration of a morphogen to the transplant
5 tissue.
- 1 35. The method of claim 34 wherein said morphogen is
2 provided to said tissue prior to transplantation.
- 1 36. The method of claim 34 wherein said morphogen is
2 provided to said transplant tissue prior to removal
3 of said tissue from the donor.
- 1 37. The method of claim 35 wherein said tissue is a
2 synthetic tissue.
- 1 38. The method of claim 37 wherein said synthetic
2 tissue comprises proliferating hepatocytes disposed
3 on a biocompatible acellular matrix.

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- 1 39. The method of claims 31 or 34 wherein said step of
2 providing a therapeutically effective morphogen
3 concentration is performed by administering a
4 morphogen to said tissue or transplant locus.
- 1 40. The method of claims 31 or 34 wherein said step of
2 providing a therapeutically effective morphogen
3 concentration is performed by administering a
4 morphogen-stimulating agent to said tissue or
5 transplant locus.
- 1 41. The method of claim 1, 2, 19, 28, 31 or 34 wherein
2 said morphogen comprises an amino acid sequence
3 sharing at least 70% homology with one of the
4 sequences selected from the group consisting of:
5 OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx),
6 GDF-1(fx) and 60A(fx).
- 1 42. The method of claim 41 wherein said morphogen
2 comprises an amino acid sequence sharing at least
3 80% homology with one of the sequences selected
4 from the group consisting of: OP-1, OP-2, CBMP2,
5 Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A(fx).
- 1 43. The method of claim 42 wherein said morphogen
2 comprises an amino acid sequence having greater
3 than 60% amino acid identity with the sequence
4 defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
- 1 44. The method of claim 43 wherein said morphogen
2 comprises an amino acid sequence having greater
3 than 65% amino acid identity with the sequence
4 defined by residues 43-139 of Seq. ID No. 5 (hOP1.)

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- 1 45. The method of claim 44 wherein said morphogen
2 comprises an amino acid sequence defined by
3 residues 43-139 of Seq. ID No. 5 (hOP1), including
4 allelic and species variants thereof.
- 1 46. The method of claim 1, 2, 19, 28, 31 or 34 wherein
2 said morphogen is provided in its pro form.
- 1 47. The method of claim 45 wherein the morphogen is
2 provided in its pro form.
- 1 48. The method of claim 47 wherein said morphogen
2 comprises an amino acid sequence defined by
3 residues 30-431 of Seq. ID No. 16.
- 1 49. A method for correcting a liver function deficiency
2 in a mammal, the method comprising the step of :
3
4 a) attaching cells to a biocompatible, acellular
5 matrix to create a cell-matrix structure, the
6 matrix being suitable for cellular attachment,
7 proliferation and ingrowth, and said cells being
8 capable of expressing one or more proteins in vivo
9 to correct said liver function deficiency; and
10
11 b) implanting said cell-matrix structure,
12 together with a therapeutically effective
13 concentration of a morphogen, in said mammal.
- 1 50. A gene therapy treatment method for correcting a
2 protein deficiency in a mammal, the method
3 comprising the step of:
4

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5 a) attaching cells to a biocompatible, acellular
6 matrix to create a cell-matrix structure, the
7 matrix being suitable for cellular attachment,
8 infiltration, proliferation and differentiation,
9 and said cells being capable of expressing one or
10 more proteins in vivo to correct said protein
11 deficiency; and
12

13 b) implanting said cell-matrix structure,
14 together with a therapeutically effective
15 concentration of a morphogen, in said mammal.
16

1 51. The method of claim 49 or 50 wherein said morphogen
2 is adsorbed to a surface of said matrix.

1 52. The method of claim 49 or 50 comprising the
2 additional step of stimulating proliferation of
3 said cells prior to implantation.

1 53. The method of claim 52 wherein said cells are
2 stimulated by exposure to a morphogen.

1 54. The method of claim 49 or 50 wherein said cells
2 comprise foreign genetic material.

1 55. The method of claim 49 or 50 wherein said cells are
2 allogenic.

1 56. the method of claim 49 or 50 wherein said matrix is
2 in vivo biodegradable.

1 57. The method of claim 49 or 50 wherein said matrix is
2 derived from organ-specific tissue.

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- 1 58. The method of claim 49 or 50 wherein said matrix
2 comprises a synthetic polymeric material.
- 1 59. The method of claim 58 wherein said polymeric
2 material comprises polylactic acid, polybutyric
3 acid, polyglycolic acid, polyanhydride, or
4 copolymers thereof.
- 1 60. The method of claim 49 or 50 wherein said matrix
2 comprises one or more tissue-derived structural
3 molecules.
- 1 61. The method of claim 60 wherein said matrix
2 comprises hyalurinc acid, laminin or collagen.
- 1 62. The method of claim 49 or 50 wherein said matrix
2 further comprises cell attachment factors.
- 1 63. The method of claim 62 wherein said cell attachment
2 factors include glycosaminoglycans, proteoglycans.
- 1 64. The method of claim 49 or 50 wherein said cell-
2 matrix structure is implanted at a liver-specific
3 tissue locus.
- 1 65. The method of claim 49 or 50 wherein said cell-
2 matrix structure is implanted at an extra-hepatic
3 tissue locus.
- 1 66. A composition for correcting a liver function
2 deficiency in a mammal, the composition comprising:
3
4 a) cells capable of expressing one or more
5 protein in vivo to correct said liver function
6 deficiency;

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- 7
8 b) a biocompatible, acellular matrix having a
9 three-dimensional structure suitable for the
10 attachment, infiltration, differentiation and
11 proliferation of said hepatocytic cells; and
12
13 c) a morphogen, such that said cells, when
14 attached to said matrix and stimulated by said
15 morphogen, are capable of correcting said liver
16 function deficiency when implanted in said mammal.
17

- 1 67. A composition useful in a gene therapy protocol to
2 correct a protein deficiency in a mammal, the
3 composition comprising:
4
5 a) cells capable of expressing one or more
6 protein in vivo to correct said protein deficiency;
7
8 b) a biocompatible, acellular matrix having a
9 three-dimensional structure suitable for the
10 attachment, infiltration, differentiation and
11 proliferation of said cells; and
12
13 c) a morphogen, such that said cells, when
14 attached to said matrix and stimulated by said
15 morphogen, are capable of expressing one or more
16 proteins to correct said protein deficiency when
17 implanted in said mammal.

- 1 68. The composition of claim 66 or 67 wherein said
2 cells comprise foreign genetic material.
- 1 69. The composition of claim 67 wherein said foreign
2 genetic material encodes said correcting proteins.

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- 1 70. The composition of claim 66 or 67 wherein said
2 cells are allogenic.
- 1 71. The composition of claim 66 or 67 wherein said
2 matrix is in vivo biodegradable.
- 1 72. The composition of claim 66 or 67 wherein said
2 matrix is derived from organ-specific tissue.
- 1 73. The composition of claim 72 wherein said matrix is
2 derived from hepatic tissue.
3
- 1 74. The composition of claim 66 or 67 wherein said
2 matrix comprises a synthetic polymeric material.
- 1 75. The composition of claim 74 wherein said polymeric
2 material comprises polylactic acid, polybutyric
3 acid, polyglycolic acid, polyanhydride, or
4 copolymers thereof.
- 1 76. The composition of claim 66 or 67 wherein said
2 matrix comprises a tissue-derived structural
3 molecule.
- 1 77. The composition of claim 76 wherein said structural
2 molecule includes collagen, laminin or hyaluronic
3 acid.
- 1 78. The composition of claim 66 or 67 wherein said
2 matrix further comprises cell attachment factors.

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- 1 79. The composition of claim 78 wherein said cell
2 attachment factors include glycosaminoglycans or
3 proteoglycans.
- 1 80. The invention of claim 49, 50, 66 or 67 wherein
2 said morphogen comprises an amino acid sequence
3 sharing at least 70% homology with one of the
4 sequences selected from the group consisting of:
5 OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx),
6 GDF-1(fx) and 60A(fx).
- 1 81. The invention of claim 80 wherein said morphogen
2 comprises an amino acid sequence sharing at least
3 80% homology with one of the sequences selected
4 from the group consisting of: OP-1, OP-2, CBMP2,
5 Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx), and 60A(fx).
- 1 82. The invention of claim 81 wherein said morphogen
2 comprises an amino acid sequence having greater
3 than 60% amino acid identity with the sequence
4 defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
- 1 83. The invention of claim 82 wherein said morphogen
2 comprises an amino acid sequence having greater
3 than 65% amino acid identity with the sequence
4 defined by residues 43-139 of Seq. ID No. 5 (hOP1.)
- 1 84. The invention of claim 83 wherein said morphogen
2 comprises an amino acid sequence defined by
3 residues 43-139 of Seq. ID No. 5 (hOP1), including
4 allelic and species variants thereof.
- 1 85. The invention of claim 49, 50, 66 or 67 wherein
2 said morphogen is provided in its pro form.

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- 1 86. The invention of claim 84 wherein the morphogen is
2 provided in its pro form.
- 1 87. The invention of claim 86 wherein said morphogen
2 comprises an amino acid sequence defined by
3 residues 30-431 of Seq. ID No. 16.
- 1 88. The use of a morphogen in the manufacture of a
2 pharmaceutical for enhancing the level of depressed
3 liver function or for maintaining normal liver
4 function following tissue injury or disease.
- 1 89. The use of a morphogen in the manufacture of a
2 pharmaceutical to regenerate lost or damaged
3 hepatic tissue or to enhance integration of a liver
4 transplant.
- 1 90. The use of a morphogen in the manufacture of an
2 implantable, proliferating cellular device to
3 correct a liver function deficiency or protein
4 deficiency in a mammal.
- 1 91. The use according to claim 88, 89 or 90 wherein
2 said morphogen comprises an amino acid sequence
3 sharing at least 70% homology with one of the
4 sequences selected from the group consisting of:
5 OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx),
6 GDF-1(fx) and 60A(fx).
- 1 92. The use according to claim 88, 89 or 90 wherein
2 said morphogen comprises an amino acid sequence
3 having greater than 60% amino acid identity with
4 the sequence defined by residues 43-139 of Seq. ID
5 No. 5 (hOP1.)

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- 1 93. The use according to claim 88, 89 or 90 wherein
2 said morphogen comprises an amino acid sequence
3 defined by residues 43-139 of Seq. ID No. 5 (hOP1),
4 including allelic and species variants thereof.
- 1 94. A kit for detecting a reduced liver function or
2 hepatocellular injury in a mammal, or for
3 evaluating the efficacy of a therapy for treating a
4 malady associated with reduced liver function or
5 hepatocellular injury in a mammal, the kit
6 comprising:
7 c) means for capturing a cell or body fluid
8 sample obtained from a mammal;
9 b) a binding protein that interacts specifically
10 with a morphogen in said sample so as to form a
11 binding protein-morphogen complex;
12 c) means for detecting said complex.
- 1 95. The kit of claim 94 which said binding protein has
2 specificity for an epitope defined by part or all
3 of the pro region of a morphogen.
- 1 96. A method for detecting a reduced liver function or
2 hepatocellular injury in a mammal, or for
3 evaluating the efficacy of a therapy for treating a
4 malady associated with reduced liver function or
5 hepatocellular injury in a mammal, the method
6 comprising the step of:
7 detecting fluctuations in the physiological
8 concentration of a morphogen or a morphogen
9 antibody titer present in the serum or peritoneal
10 fluid of said mammal, said fluctuations being
11 indicative of an increase in hepatic cell death.

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1 97. The invention of claim 1, 2, 28, 31, 49, 50, 66,
2 67, 88, 89 or 90 wherein said morphogen comprises a
3 dimeric protein species complexed with a peptide
4 comprising a pro region of a member of the
5 morphogen family, or an allelic, species or other
6 sequence variant thereof.

1 98. The invention of claim 97 wherein said dimeric
2 morphogen species is noncovalently complexed with
3 said peptide.

1 99. The invention of claim 97 wherein said dimeric
2 morphogen species is complexed with two said
3 peptides.

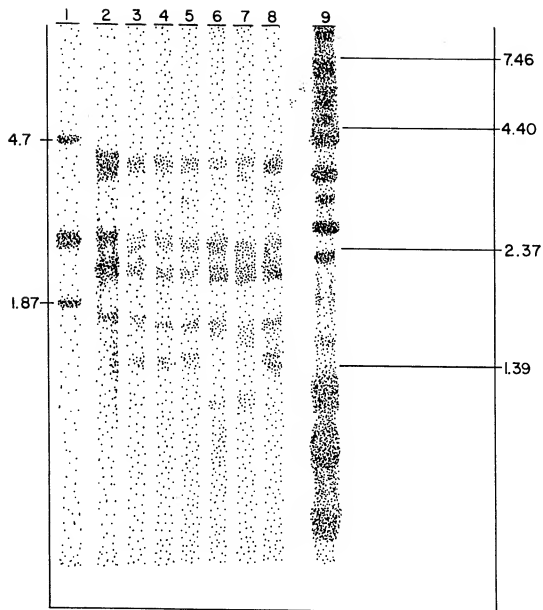
1 100. The invention of claim 97 wherein said peptide
2 comprises at least the first 18 amino acids of a
3 sequence defining said pro region.

1 101. The invention of claim 100 wherein said peptide
2 comprises the full length form of said pro region.

1 102. The invention of claim 97 wherein said peptide
2 comprises a nucleic acid that hybridizes under
3 stringent conditions with a DNA defined by nucleotides
4 136-192 of Seq. ID No. 16, or nucleotides 157-211 of
5 Seq. ID No. 20.

1 103. The invention of claim 97 wherein said complex is
2 further stabilized by exposure to a basic amino acid, a
3 detergent or a carrier protein.

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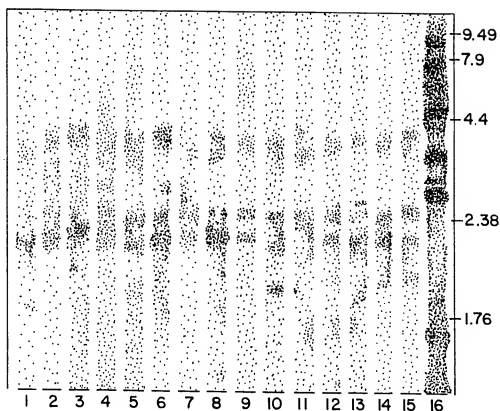
*Fig. 1*

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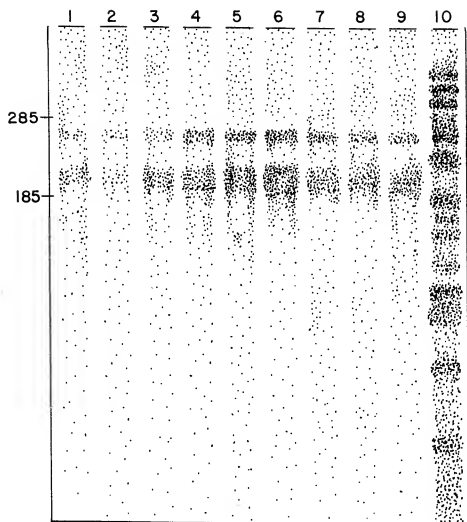


Fig. 2

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*Fig. 3*

4/6

*Fig. 4*

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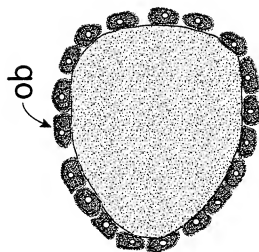


Fig. 5B

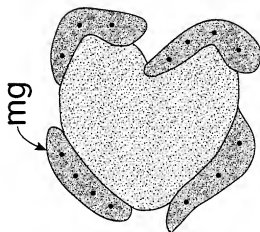


Fig. 5A

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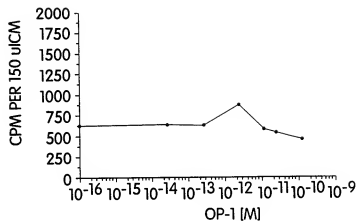


Fig. 6A

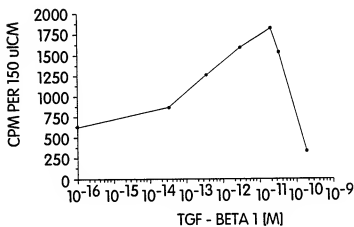


Fig. 6B

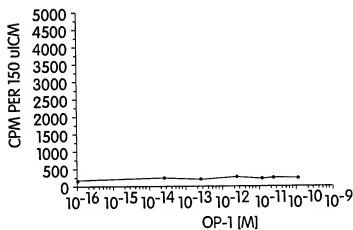


Fig. 6C

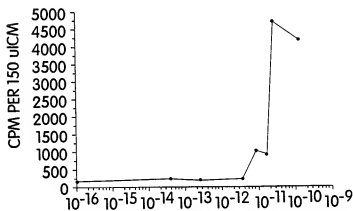


Fig. 6D



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 37/02	A3	(11) International Publication Number: WO 94/06449 (43) International Publication Date: 31 March 1994 (31.03.94)									
<p>(21) International Application Number: PCT/US93/08808</p> <p>(22) International Filing Date: 16 September 1993 (16.09.93)</p> <p>(30) Priority data:</p> <table border="0"> <tr> <td>946,238</td> <td>16 September 1992 (16.09.92)</td> <td>US</td> </tr> <tr> <td>029,335</td> <td>4 March 1993 (04.03.93)</td> <td>US</td> </tr> <tr> <td>040,510</td> <td>31 March 1993 (31.03.93)</td> <td>US</td> </tr> </table> <p>(71) Applicant: CREATIVE BIOMOLECULES, INC. [US/US]; 45 South Street, Hopkinton, MA 01748 (US).</p> <p>(72) Inventors: KUBERASAMPATH, Thangavel ; Six Spring Street, Medway, MA 02053 (US). RUEGER, David, C. ; 19 Downey Street, Hopkinton, MA 01748 (US). OPPER-MANN, Hermann ; 25 Summer Hill Road, Medway, MA 02053 (US). PANG, Roy, H., L. ; 15 Partridge Road, Etna, NH 03750 (US). COHEN, Charles, M. ; 98 Winthrop Street, Medway, MA 02053 (US). OZKAYNAK, Engin ; 44 Purdue Drive, Milford, MA 01757 (US). SMART, John, E. ; 50 Meadow Brook Road, Weston, MA 02193 (US).</p>		946,238	16 September 1992 (16.09.92)	US	029,335	4 March 1993 (04.03.93)	US	040,510	31 March 1993 (31.03.93)	US	<p>(74) Agent: KELLEY, Robin, D.; Testa, Hurwitz & Thibault, Exchange Place, 53 State Street, Boston, MA 02109 (US).</p> <p>(81) Designated States: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p> <p>(88) Date of publication of the international search report: 1st September 1994 (01.09.94)</p>
946,238	16 September 1992 (16.09.92)	US									
029,335	4 March 1993 (04.03.93)	US									
040,510	31 March 1993 (31.03.93)	US									

(54) Title: MORPHOGEN-INDUCED LIVER REGENERATION**(57) Abstract**

Disclosed are therapeutic treatment methods, compositions and devices for maintaining liver function in a mammal, including means for regenerating lost or damaged hepatic tissue, means for enhancing viability and integration of hepatic tissue and organ transplants, and means for correcting liver function deficiencies, including means for enhancing diminished liver function due to tissue injury or disease. The methods, compositions and devices on this invention all provide a therapeutically effective morphogen concentration to the hepatic cells to be treated. Also disclosed are methods and compositions useful in a gene therapy or drug delivery protocol for correcting a protein deficiency in a mammal.

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 93/08808

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,92 15323 (CREATIVE BIOMOLECULES) 17 September 1992 cited in the application see page 6, line 27 - page 7, line 8 see page 14, line 13-25; claims ---	1-91
P,X, Y	WO,A,93 04692 (CREATIVE BIOMOLECULES) 18 March 1993 see page 1-4 see page 6, line 26 - page 7 see page 80, line 15 - page 83, line 4; claims 1,26-32,49,55 ---	1-91
X	WO,A,92 09301 (THE AMERICAN NATIONAL RED CROSS) 11 June 1992 see page 9, line 8-15 see page 15, line 15-20; claims 1,4,8,11,15 --- -/-	1,5,17, 19,35

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

19 July 1994

Date of mailing of the international search report

25.07.94

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INTERNATIONAL SEARCH REPORT

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C/(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	WO,A,89 10409 (GENETICS INSTITUTE, INC.) 2 November 1989 see page 8, line 1-7; claims -----	1-91
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/08808

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although some claims are directed to a method of treatment of the human body the search has been carried out. It was based on the alleged effects of the compositions.
2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The expression "morphogen" is not sufficient to characterize specific chemical compounds. In view of the extremely large number of substances encompassed by this term, the search had to be limited to the general concept and to the specific compounds mentioned in the claims ... (please see annex
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

.... and in the pharmacological examples (see PCT, art. 6; Guidelines for Examination in the E.P.O., Part B, Chapter II.7, last sentence and Chapter III.3.7).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 93/08808

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP-A-	0575555
			29-12-93
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		EP-A-	0601106
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		CA-A-	2116560
		EP-A-	0601129
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		CA-A-	2097063
		EP-A-	0564502
			13-10-93
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		AU-A-	3448789
		EP-A-	0408649
		JP-T	3503649
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		US-A-	5187076
			16-02-93